Prolonged Drug Delivery from a Polymeric Fibre Device for the Treatment of Periodontal Disease

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A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfillment of the requirements for the degree of Master of Pharmacy

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Johannesburg, 14 October 2011

DECLARATION

I, Deanne Mary Graham Hazle declare that this dissertation is my own work. It is being submitted for the degree of Master of Pharmacy in the Faculty of Health Sciences in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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This 14th of October 2011

RESEARCH OUTPUTS

1. Posters Presented at Conferences

Flexural and extensibility testing of co-blended amphiphilic triethanolamine and transesterified epichlorohydrin-based alginate fibres, Deanne Hazle, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit and Michael P. Danckwerts, 29th Annual Conference of the Academy of Pharmaceutical Sciences of South Africa, Hunters Rest Resort, Magaliesburg, Johannesburg, South Africa, September 22-26, 2008 (Appendix A)

Characterisation of co-blended amphiphilic transesterified epichlorohydrin-based alginate fibres for oramucosal delivery, Deanne Hazel, Viness Pillay, Yahya E. Choonara and Lisa C. du Toit, International Student Congress of Medical Sciences (ISCOMS) 2009, University Medical Centre in Groningen, The Netherlands, June 2-5, 2009 (Appendix B)

2. Podium presentations

Evaluation of the physicochemical and physicomechanical properties of optimized ciprofloxacin- and diclofenac-loaded co-blended alginate fibers for oramucosal delivery, Deanne Hazle, Viness Pillay, Yahya E. Choonara, Lisa C. du Toit, Michael P. Danckwerts and Sandy van Vuuren, to be presented at the ICPPS Conference, Academy of Pharmaceutical Sciences Young Scientists Competition, September 25-27, 2011 (Appendix C)

3. Publications submitted

nanoTensileTM Analysis of Co-Blended Amphiphilic Tranesterified-Based Alginate Fibres, Deanne Hazle, Viness Pillay, Pradeep Kumar, Yahya E. Choonara, Lisa C. Du Toit. Submitted to Journal of Material Science.

ABSTRACT

Periodontal disease describes a chronic bacterial infection affecting the gums and bone supporting the teeth. Bacteria present in plaque produce toxins, which lead to a cascade of inflammatory events that if left untreated may lead to permanent tooth loss. A periodontal pocket forms when the free gingiva moves away from the tooth surface.

Periodontal disease is prevalent worldwide and has risk factors such as HIV and diabetes with possible links to socio-economic status. This places a large portion of the South African population at risk in an already burdened health care system.

Scaling and root planning (SRP) forms the keystone of periodontal therapy, involving the removal of calculus and plaque. Multiple clinical trials have proved SRP leads to improved clinical outcomes. However, it often leaves behind microorganisms leading to recolonisation. Administration of pharmacological therapy is used in combination with SRP delivering one or more drugs. Subgingival treatment of periodontal disease involves the placement of a drug delivery device within the periodontal pocket releasing model drugs over a prolonged period of time. Targeted drug delivery devices have been the focus of periodontal research over the past two decades. To date there are no commercially available local drug delivery devices in South Africa for the treatment of periodontal disease.

The aim of this study was to design, formulate and evaluate (*in vitro*) a novel polymeric fibre system to locally deliver an antimicrobial and an anti-inflammatory drug over 10 days to the periodontal pocket for the treatment of periodontal disease. The design of a flexible fibre would easily fit within the periodontal pocket evenly delivering the model drugs to the affected site. Alginate combined with glycerol was crosslinked with barium cations forming a monolithic fibre incorporating ciprofloxacin and diclofenac sodium, as the model antimicrobial and anti-inflammatory agents respectively. A 3-Factor Box-Behnken Design was employed to statistically optimise the fibres according to their tensile properties and drug release. The optimised formulation (3.14%$^{w}/_{v}$ alginate, 22.54mL glycerol and 10.00$^{w}/_{v}$ barium chloride) was evaluated for its drug release and hydration behaviour at pH 4 and 6.8, vibrational transitions and tensile properties as well as antimicrobial assays, characterising the *in vitro* behaviour of the device. The pH of the periodontal pocket varies from pH 2-9. Crosslinked alginate matrices demonstrate pH-responsive behaviour, therefore the polymeric fibre device was tested at pH 4 and 6.8. Drug release at pH 4 occurred as a result of drug diffusing through the polymeric fibres. However, at pH 6.8 the disruption of the fibre structure led to drug release as a consequence of the swelling and erosion of the matrix. Ciprofloxacin was sufficiently released from the drug-loaded fibres inhibiting growth of *Escherichia coli*, *Enterococcus faecalis* and *Streptococcus mutans* over 10 days. The physicomechanical and physicochemical properties were related to the degree of crosslinking, the effect of the plasticiser and the interaction of formulation components. The polymeric fibre device formed a strong yet flexible biodegradable matrix which simultaneously released an antimicrobial and anti-inflammatory agents in phosphate buffer solution pH 6.8 over 10 days. The promising *in vitro* results advocate for further analysis of the fibres.

ACKNOWLEDGEMENTS

"One may walk over the highest mountains one step at a time."

John Wanamaker

Completing my masters degree has been the mountain I have been climbing for quite some time now. My journey would have been that much more difficult if it had not been for the help of colleagues, supervisors, family and friends who took so many of the steps by my side.

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I am blessed with a wonderful family who have walked each step of my journey beside me. I would never have managed to persevere through the difficult times I encountered without the loving support of my mom and dad as well as my brothers Craig and Rogan. Thank you for always believing in me. To my extended family and friends, I appreciate all the words of encouragement and love I have always felt.

To my dearest love, Simon, I would be lost without you. You are a husband in a million. For the many meals you cooked, for all the household chores you took control of, for your unwavering belief in me and for your continual love, I will be eternally grateful. I will love you always.

DEDICATION

I dedicate this dissertation to my late mother, Glynda Hazle, who passed away on the 23rd of August 2010. Your endless support in everything I attempt and unconditional love has made me into the person I am. You gave so selflessly to your family, listening to my every story and never failing to say “I know you can De.” I will miss you always, Mom!

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CHAPTER 1

INTRODUCTION

1.1. Defining and Classifying Periodontal Disease

Periodontal disease (PD) describes a chronic bacterial infection affecting the gums and bone supporting the teeth. It refers to a broad category of oral manifestations that can be further classified as the following: gingival diseases, chronic periodontitis, aggressive periodontitis, periodontitis as a manifestation of systemic diseases, necrotising periodontal diseases, abscesses of the periodontium, periodontitis associated with endodontic lesions and developmental or acquired deformities and conditions (Figure 1.1) (Armitage, 1999). PD is prevalent worldwide, with 10-15% of adults presenting with advanced signs of the disease, which is periodontal pockets greater than 6mm in depth (Perterson and Ogawa, 2005).

Poor oral hygiene can lead to gingivitis, characterised by red swollen gums that bleed easily. Gingivitis affects a large portion of the world's population (Pihlstrom *et al*., 2005) and if left untreated may advance to periodontitis (Figure 1.2). Bacteria present in plaque, produce toxins that lead to a cascade of inflammatory events, resulting in the breakdown of tissues and bones supporting the teeth, and the formation of a pocket. Pockets are formed as the gum separates from the tooth forming a reservoir that may support the growth of microorganisms. Pocket depth is a clinical measurement indicative of the severity of periodontitis. As more tissue and bone are destroyed, permanent tooth loss may result. The formation of plaque leading to development of gingivitis and the subsequent progression to periodontitis is outlined in Figure 1.2.

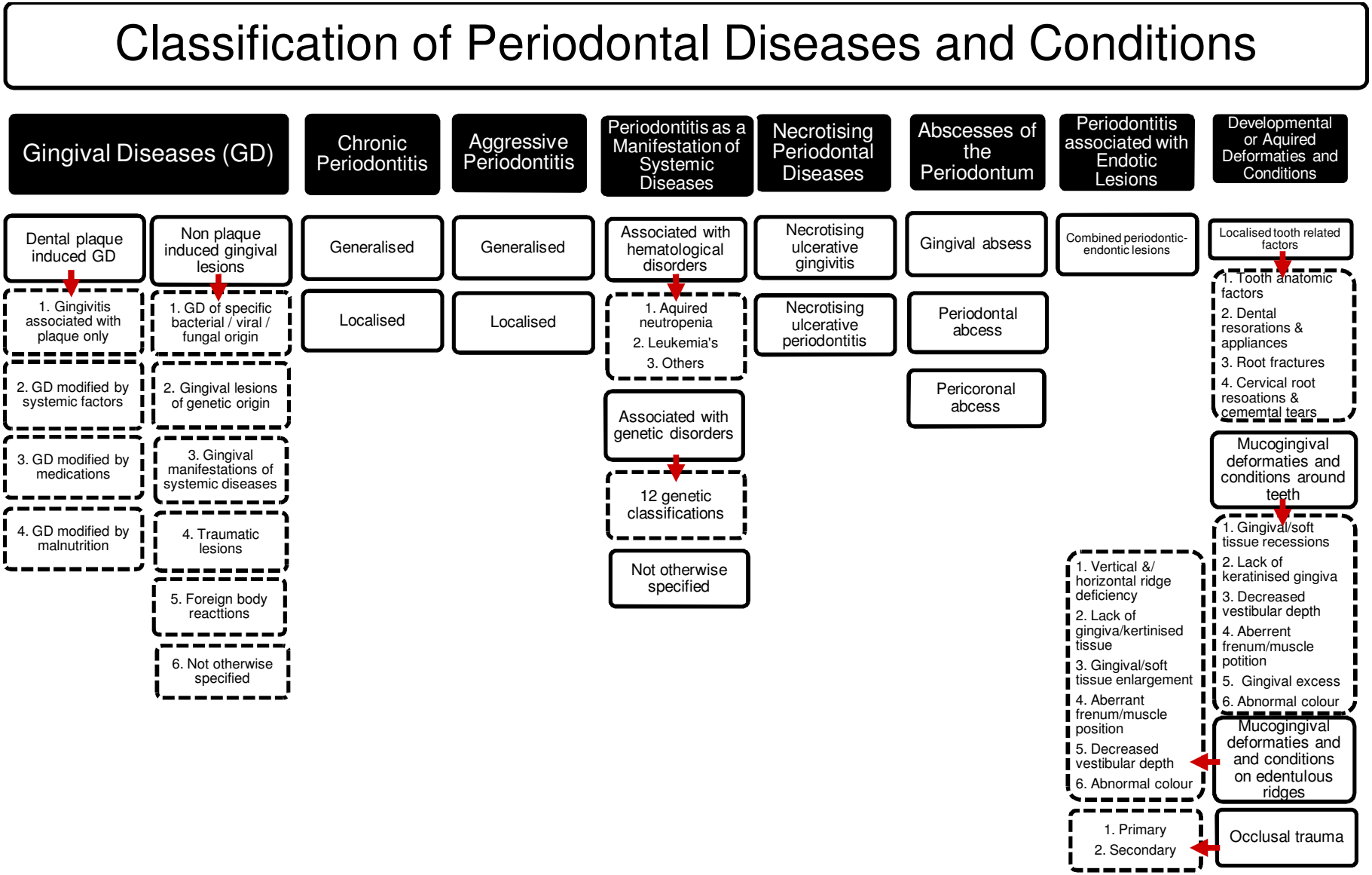


Figure 1.1: Classification of PD and conditions as decided upon at the international workshop held in October/November 1999 (Adapted from Armitage, 1999)

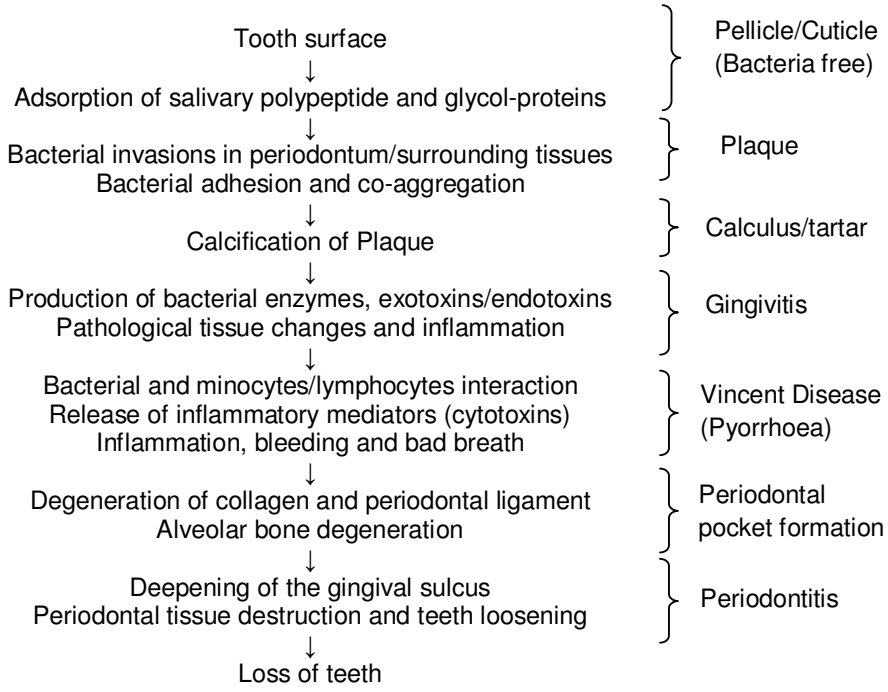


Figure 1.2: Flow chart of the possible pathogenic mechanism of PD (Jain *et al.*, 2008)

The two distinctive features of PD are the presence of microorganisms and inflammatory mediators, as presented in Figure 1.2. The interaction between bacteria and the immunoinflammatory response may lead to periodontal tissue destruction (Vardar *et al.*, 2003) which forms the rationale for the treatment with anti-inflammatory and/or antimicrobial agents.

Normally healthy oral mucosa is host to both aerobic and anaerobic bacteria forming a symbiotic relationship (Pihlstrom *et al.*, 2005). It is estimated that 200–500 microorganisms are present in the periodontal pocket (Southard and Godowski, 1998) with a greater number of Gram-negative and anaerobic bacteria (Wayne *et al.*, 2001; Jain *et al.*, 2008). Causative pathogens include: *Porphyromonas gingivalis* (*P. gingivalis*), *Tannerella forsythensis* (*T. forsythensis*), *Treponema denticola* (*T. denticola*), *Actinobacillus actinomycetemcomitans* (*A. actinomycetemcomitans*) and are accompanied by the release of bacterial leukotoxins, collenagenases, fibrionlysins and other proteases (Pihlstrom *et al.*, 2005). The use of antimicrobial medication in periodontal therapy is due to the microbial aetiology of inflammatory PD (Research, Science and Therapeutic Committee of the American Academy of Periodontology, 2004).

Following local tissue destruction phospholipids present in the cell membrane, through the enzymatic action of phospholipase, lead to the formation of arachidonic acid (Heasman, 1988). Furthermore, arachidonic acid is metabolised into prostaglandins, prostacyclin and

thromoboxane via the cyclooxygenase pathway or leukotrienes via the lipoxygenase pathway, as illustrated in Figure 1.3. The cyclooxygenase enzyme (COX) is present in two is forms as COX-1, antithromogenic and cytoprotective, and COX-2, inducing the production of prostaglandins (Vardar *et al*., 2003). Gingival tissue concentration of prostaglandin (PG) E_2 and leukotriene B_4 are increased in PD with alveolar bone destruction (Requirand *et al*., 2000). Therefore, a potential mechanism for blocking PD progression may depend on use of non-steroidal anti-inflammatory drugs (NSAIDs), through regulation of arachidonic acid metabolites alveolar bone resorption may be suppressed (Salvi and Lang, 2005).

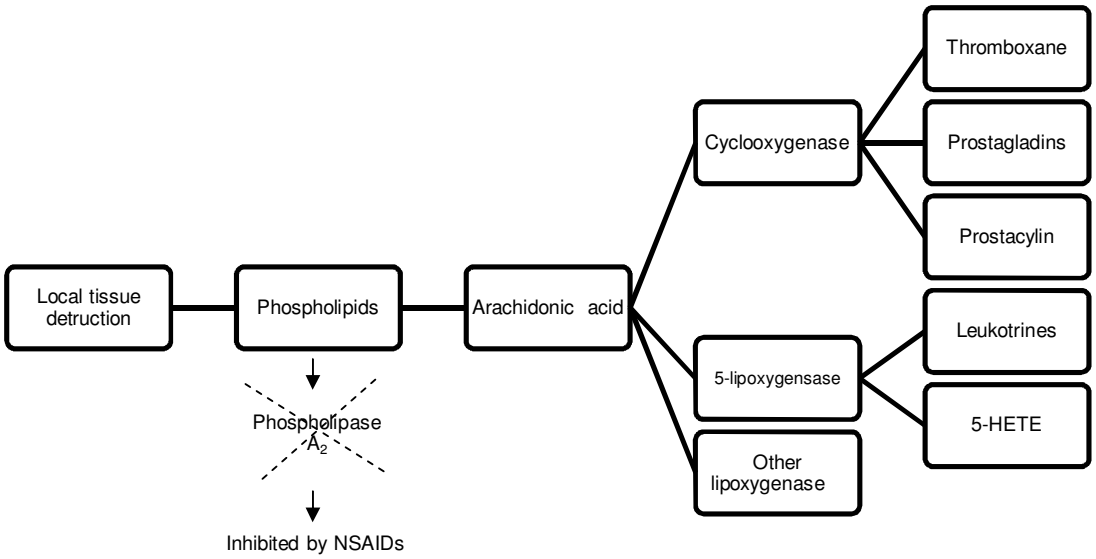


Figure 1.3: Simplified schematic of arachidonic acid metabolism indicating NSAIDs inhibiting phospholipase A_2

1.2. Rationale for the Study

Delivery of therapeutic agents to the periodontium can be achieved through local or systemic administration. The success of the treatment is largely dependent on the environments in which the agent is administered, the mode of administration, the length of time that the therapeutic agent remains in the periodontal pocket and the type of therapeutic agent administered (Addy and Fugit, 1989; Goodson, 1994). There are three possible environments to administer therapeutic agents which deliver the drug to the periodontium, namely the supragingival or subgingival regions or systemically (Goodson, 1994). Local drug delivery includes drugs that are administered to the supragingival and subgingival environments. Systemic drugs are administered via the oral route, absorbed in the gastrointestinal tract and enter the systemic circulation, where the drugs are distributed

throughout the body. The supragingival environment refers to saliva, supragingival plaque, tongue, tonsils and oral mucosa, where therapeutic agents are normally administered as mouthwashes. The subgingival environment includes the gingival fluid and periodontal soft tissue (Goodson, 1994) with several drug therapies being developed for placement in the periodontal pockets, usually releasing the therapeutic agents over an extended period of time. Ryan (2005) summarised systemically delivered antimicrobial agents as well as supragingival and subgingival antimicrobial formulations, comparing the ability of the antimicrobial agent to penetrate deep within the periodontal pocket as well as the concentration of drug and duration of treatment (Table 1.1)

Table 1.1: Administration of antimicrobial agents to the periodontal pocket via four modes of application (Ryan, 2005)

Objective	*Mouthrinse or toothpaste*	*Local irrigation*	*Systemic delivery*	*Controlled delivery*
Reach pocket depth of 4mm	Poor	Good	Good	Excellent
Adequate concentration	Poor	Good	Fair	Excellent
Adequate duration	Poor	Poor	Fair	Good

Systemic drug delivery has traditionally been the preferred route of drug administration and refers to orally administered drugs. This popular route is used for systemic delivery of antimicrobials and anti-inflammatory agents for the treatment of PD. The systemic route of delivery is easy and convenient to use as well as ensuring even distribution of the drug to multiple sites of infection (Research, Science and Therapeutic Committee of the American Academy of Periodontology, 2004). Disadvantages of systemic drug delivery include reduced patient compliance (Goodson, 1994; Loesche, 1998) and increased risk of adverse drug reactions (Soskolne, 1997; Research, Science and Therapeutic Committee of the American Academy of Periodontology, 2004) as well as leading to the emergence of resistant strains (Goodson, 1994). Peak concentrations of typical systemically administered antimicrobials are 3µg/mL in the gingival fluid and blood, however levels of between 1-16µg/mL are necessary to inhibit the growth of microorganisms (Goodson, 1994).

Site-specific drug delivery leads to administration of drugs via mucosal linings, namely nasal, rectal, vaginal, ocular and oral. The advantages of delivery through these transmucosal routes are that the dosage by-passes first pass metabolism in the liver, avoids presystemic elimination and directly delivers the drug to the systemic circulation (Shojaei, 1998; Hearnden *et al.*, 2011). The aim of local drug delivery in PD is to achieve higher concentrations of therapeutic agents within the periodontal pocket over a predetermined period of time.

Addy and Fugit (1989) differentiated the local drug delivery to the oral cavity according to the vehicle used in the delivery devices based on the expected time which the therapeutic agent would remain in the mouth as follows:

a) Short-term (seconds to minutes): examples are toothpastes, mouthwashes and irrigations.
b) Medium-term (hours): examples are gels and ointments.
c) Long-term (days to weeks): examples are degradable and non-degradable sustained delivery devices.

Short to medium term delivery vehicles are used mainly in supragingival plaque control and in the prevention of gingivitis (Addy, 1994). Pitcher *et al.* (1980) noted that mouth rinsing did not penetrate periodontal pockets sufficiently, therefore limiting its use in subgingival applications.

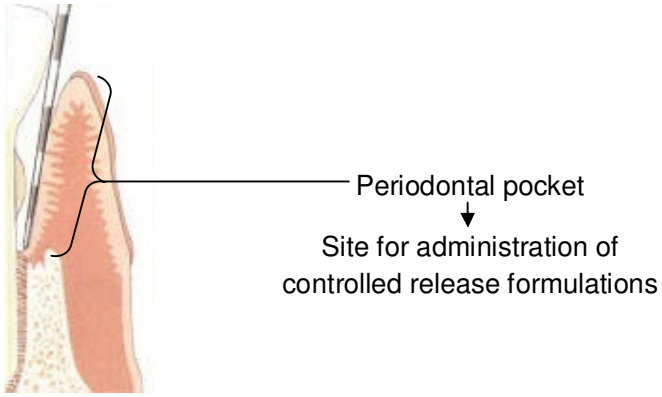


Figure 1.4: Periodontal pocket: site of implantation of sustained drug delivery devices (Adapted from Gupta, 2008)

Sustained drug delivery devices can be further subdivided into degradable and non-degradable devices. The device generally consists of a matrix within which the drug is evenly distributed. In non-degradable devices, the drug diffuses from an insoluble non-degradable polymer which needs to be removed after treatment is completed, while degradable devices release the drug via diffusion and matrix erosion and therefore do not need to be removed from the periodontal pocket (Medlicott *et al.*, 1994). Higher levels of drug in gingival fluid leading to improved clinical parameters evident with intra-pocket delivery systems (Jain *et al.*, 2008) for even distribution of drug throughout the periodontal pocket (Soskolne, 1997). Site-specific drug delivery selectively target the diseased site with superior treatment results (Research, Science and Therapeutic Committee of the American Academy of Periodontology, 2001). Furthermore, degradable devices have the added advantage of improved patient compliance as there is no need to remove the device from the periodontal pocket (Medlicott *et al.*, 1994).

Currently there are no locally-acting products on the South African market to treat PD. If a product such as this is required, special permission has to be obtained from the Medicines Control Council (MCC) of South Africa to import the product from abroad (Medicines and Related Substances Act, 1965). If the device were to be used routinely, the product would need to be registered with the MCC as a medicine. The use of existing locally delivered products is costly (Venezia and Shapira, 2003). Furthermore, the difficulty of importing these products together with the importation costs are primary reasons why local devices are not generally used for the treatment of PD in South Africa. Hence, the development of a drug delivery system within South Africa is necessary.

1.3. Aims and Objectives

The aim of this study was to design, formulate and evaluate a novel polymeric fibre device (PFD) to deliver an antimicrobial and anti-inflammatory drug over a prolonged period of time that could be used for the treatment of PD. The PFD device is placed in the periodontal pocket for site-specific delivery of ciprofloxacin (model antimicrobial agent) and diclofenac sodium (model anti-inflammatory agent). To achieve this aim, the following objectives were outlined:

1. To formulate a PFD loaded with an anti-inflammatory and antimicrobial agent.
2. To evaluate the physicochemical and physicomechanical properties of the PFD.
3. To assess the drug release behaviour of the model drugs from the PFD.
4. To employ a 3-factor Box-Benkhen statistical design to analyse the ideal experimental variables established in preliminary investigations and to determine the optimum parameters to synthesise the PFD.
5. To develop an ultra performance liquid chromatography method for the co-detection of the anti-inflammatory and antimicrobial agents.
6. To develop a quantitative method to analyse the antimicrobial efficacy of the optimised PFD.
7. To evaluate the *in vitro* antimicrobial efficacy of the optimised PFD.

1.4. Novelty of the Study

Local drug delivery systems aim to deliver drug to the site of action. The affected site in PD is the periodontal pocket, which provides an ideal environment for the administration of a drug delivery device. Numerous drug delivery systems, such as chips, gels, fibres, microparticles and strips have been formulated and tested but only a handful of these systems are now commercially available abroad and none of which are easily obtainable in South Africa.

Although numerous local delivery systems have been developed for the treatment of PD, this system is novel in the fact that:

- The PFD is a biodegradable system, intended to be placed around the tooth for even distribution of drugs within the periodontal pocket simultaneously delivering ciprofloxacin, a fluoroquinolone antibiotic, and diclofenac sodium, a nonsteroidal anti-inflammatory agent.
- An innovative method of crosslinking a co-blended polymeric plasticised matrix was employed in formulating the fibres.
- The first co-blended crosslinked glycerol and alginate PFD intended for treatment of PD.
- According to the literature surveyed, this study is one of the first investigations in formulating a local delivery device in South Africa and the first fibre developed locally intended for the treatment of periodontal disease.

1.5. Overview of the Dissertation

The content of each chapter in this dissertation is based on the aforementioned aims and objectives. A schematic, Figure 1.5, reviews the content of each chapter.

A concise review of PD followed by descriptions of the rationale and novelty of the study is given in **Chapter 1**. The literature reviewed in this chapter focuses on systemic versus local drug delivery within the periodontal pocket. The aims and objectives of the study are outlined in this chapter.

Existing chemotherapeutic approaches to the treatment of PD are reviewed in **Chapter 2,** investigating systemic and local delivery of anti-inflammatory and antimicrobial agents. The chapter examines the design of the drug delivery devices and the polymers used in controlled delivery of a drug to the periodontal pocket.

Chapter 3 reviews existing fibre formulations that have been prepared for controlled delivery of antimicrobial agents to the periodontal pocket, as well as preliminary studies in developing a suitable polymeric fibre. The effect of formulation components, identifying formulation variables and choosing a suitable antimicrobial agent are described in this chapter.

A 3-factor Box-Behnken experimental design was used for analysis and optimisation of the PFD in **Chapter 4**. The responses used as the basis for the assessment and design of the proposed fibres were; Young's modulus, ultimate strain and mean dissolution time over 10 days for the release of ciprofloxacin and diclofenac sodium. Two optimised formulations were determined and tested; following which one was selected for further analysis.

Chapter 5 analyses the physicochemical and physiocomechanical properties of the optimised PFD where both ciprofloxacin and diclofenac sodium were simultaneously loaded in one formulation. An ultra performance liquid chromatography method was developed to determine concurrent drug entrapment and release of ciprofloxacin and diclofenac sodium from the fibre. The assessment of hydration behaviour, textural properties and vibrational transitions of drug-loaded and drug-free fibres are described in this chapter. Lastly the static lattice atomistic simulations, using molecular mechanics energy relationships, elucidated the effect of formulation components and model drugs on the tensile properties of the fibre.

The antimicrobial activity analysis of optimised ciprofloxacin- and diclofenac sodium-loaded PFD is contained in **Chapter 6**. Qualitative and quantitative results were obtained employing agar diffusion assays and minimum inhibitory concentration assays respectively.

Chapter 7, the final chapter in the dissertation, contains the conclusion and future recommendations of the study.

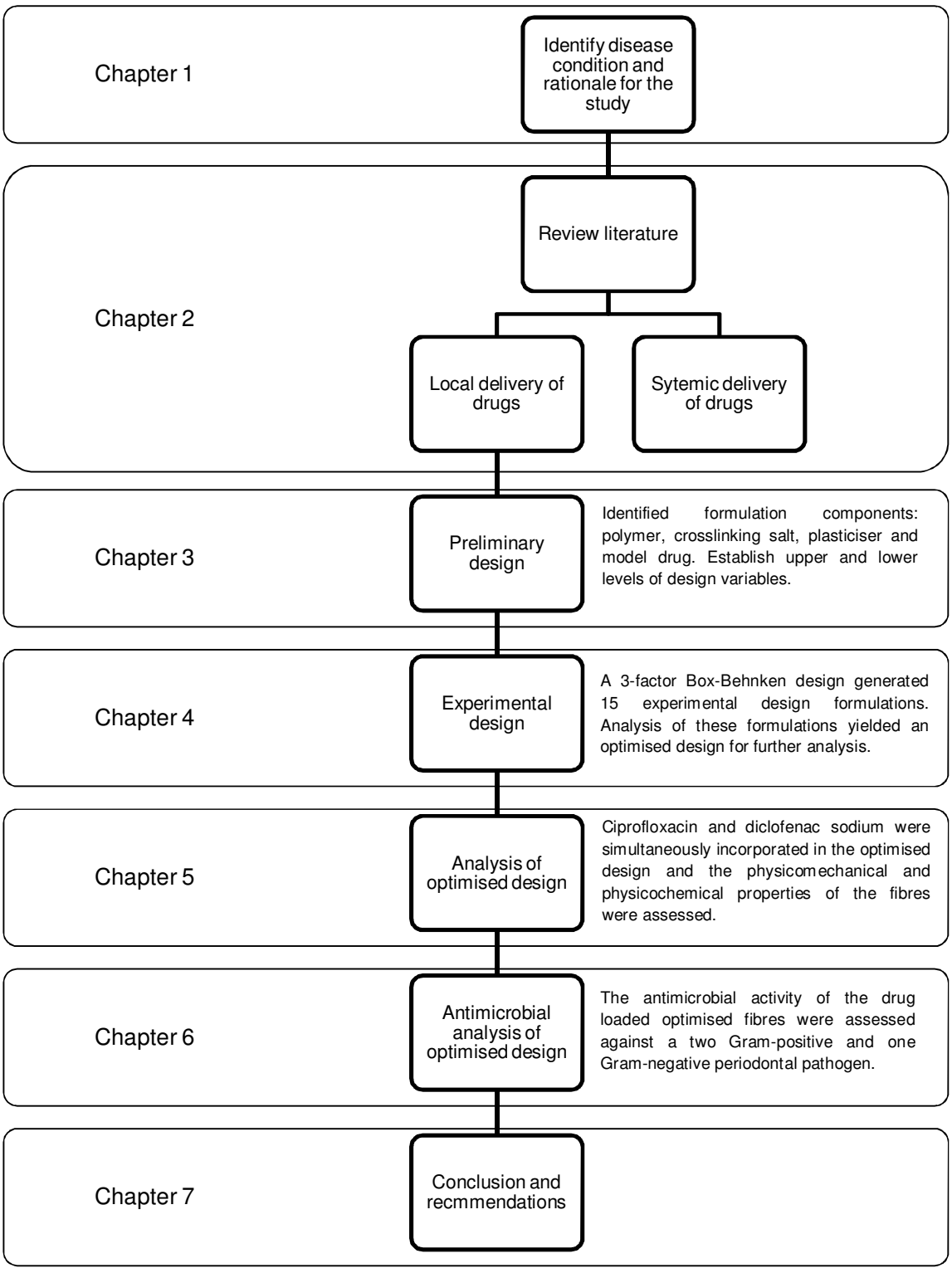


Figure 1.5: Schematic of the content contained in each chapter of the dissertation

CHAPTER 2

ANTI-INFLAMMATORY AND ANTIMICROBIAL CHEMOTHERAPEUTIC APPROACHES TO THE TREATMENT OF PERIODONTAL DISEASE

2.1. Anatomy and Physiology of the Periodontum

The Latin translation of the word periodontum is “around the tooth” and includes the gingiva and attachment apparatus. Identifying the functions and structures of the periodontum is essential to understanding the diseases affecting these structures (Bartold, 2006). A basic anatomical sketch of the periodontum is shown in Figure 2.1.

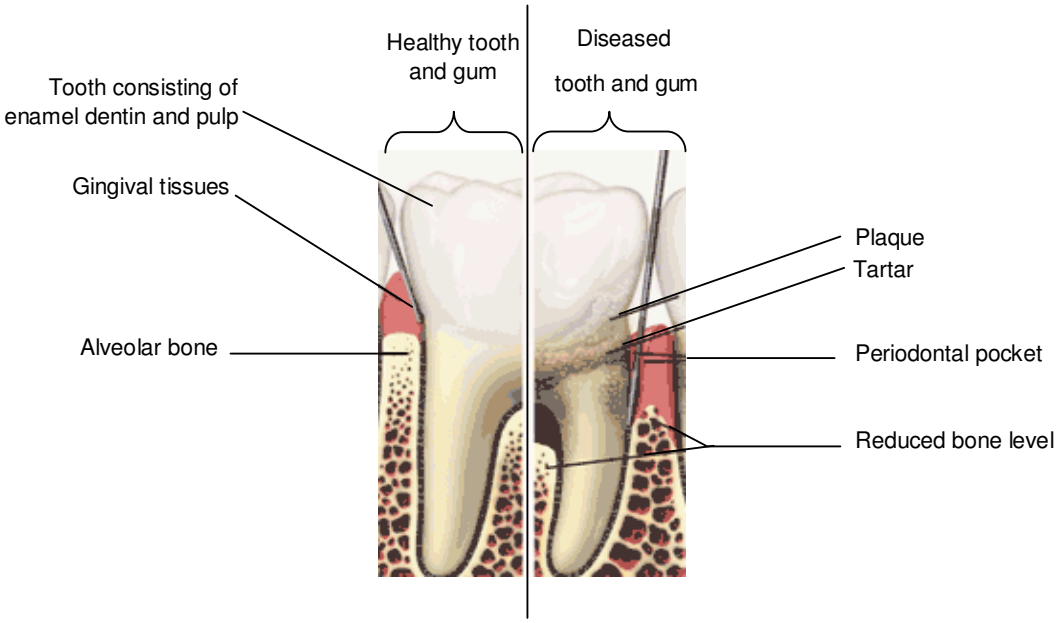


Figure 2.1: Anatomical structure of a healthy and diseased tooth and periodontum (Adapted from American Academy of Periodontology, 2011)

The gingiva is a covering that protects components of the periodontum primarily against mechanical insults such as chewing and tooth brushing. It can be anatomically subdivided into the free gingiva, attached gingiva and interdental gingiva or papilla. Covering the gingiva is a layer of stratified squamous epithelium which regularly undergoes keratinisation leading to a high degree of cell turnover. The attachment apparatus can be divided into the periodontal ligament, cementum and alveolar bone. Their primary function is that of support and securing the teeth (Hassell, 1993). Vessels of the periosteum, periodontal ligament and alveolar bone supply blood to the gingiva. The rich blood supply and high permeability of the oral mucosa leads to its popularity as a route of administration for both systemic and local drug delivery (Shojaei, 1998).

The oral mucosa has been a popular route of drug administration for a variety of systemic and local applications. The advantages and disadvantages of this route of drug administration for the purpose of treating PD are summarised in Table 2.1.

Table 2.1: Advantages and disadvantages associated with oral mucosal delivery relating to the treatment of PD (Adapted from Hearnden *et al.*, 2011; Sankar *et al.*, 2011)

Advantages	*Disadvantages*
Accessible	Permeability of oral mucosa
Highly hydrated environment for drug dissolution	Mastication and speech may dislodge device
Possible sustained delivery	Taste requires important consideration
Potential reduction in systemic side effects	Enzymes present
Less drug is incorporated within the device as targeted drug delivery reduces wastage	Small surface area available for absorption of drug
Avoids first pass metabolism	Risk of choking / swallowing device
Rich blood supply	Saliva may wash drug away

Clinical measures for the detection of periodontitis are gingival bleeding; bone loss (assessed through radiographs); probing depth of the pocket and Clinical Attachment Loss (CAL) (Research, Science and Therapeutic Committee of the American Academy of Periodontology, 2005). PD and CAL can be defined as the distance from the gingival margin and cementoenamel junction respectively, to the base of the crevice. Probing depth is rapidly measured and provides a good assessment of PD. In comparison, CAL estimates the damage to the periodontum, but is more difficult to measure (Research, Science and Therapeutic Committee of the American Academy of Periodontology, 2003).

2.2. Risk Factors

Identification of risk factors in patients is useful in determining their predisposition to developing certain conditions. Extensive research has been conducted into factors that may increase the possibility of developing PD. Review papers that summarised epidemiological studies were analysed to identify the key risk factors. Brief descriptions of the risk factors identified from these studies are given in Table 2.2.

Table 2.2: Risk factors associated with PD

Risk Factor	*Relationship with (PD)*	*Review Article*
Plaque and Oral hygiene	• Causative relationship between plaque accumulated from poor oral hygiene and gingivitis. • Weak correlation between reduction in plaque accumulation and PD.	*RST American Academy of Periodontology, 1996
Microbiota	• Gram-negative bacteria present at diseased sites*: A. actinomycetemcomitans*, *T. forsynthesis*, *P. gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Camphylobacter rectus*, *T. denticola*.	*RST American Academy of Periodontology, 1996
Smoking	• Smoking increases risk of developing PD. • PD more severe in smokers compared to non smokers.	*RST American Academy of Periodontology, 1996
Diet	• Increased risk PD associated with severe vitamin C deficiency and malnutrition.	Petersen and Ogawa, 2005 Pihlstrom *et al*., 2005
Diabetes mellitus	• Higher prevalence in patients with diabetes mellitus. • More severe periodontal infections compared to non-diabetics. • Increased risk associated with poorly controlled diabetics.	*RST American Academy of Periodontology, 1996 Petersen and Ogawa, 2005 Pihlstrom *et al*., 2005
HIV/AIDS	• More severe and unusual forms of PD with AIDS. • CD4+ counts less than 200 cells per microliter often associated with the presence of necrotizing ulcerative periodontitis.	*RST American Academy of Periodontology, 1996 Pihlstrom *et al*., 2005

*RST – Research, Science and Therapeutic Committee

The majority of the studies from which the data in Table 2.2 was obtained were conducted on patients in the USA. Studies completed in South Africa and other parts of Africa provide pertinent information regarding the prevalence of and potential risk factors predisposing parts of the population to PD. These studies relate to socio-economic variables (Gugushe, 1998; Naidoo *et al*., 2001), diabetes (Peck *et al*., 2006; Matu *et al*., 2009) and HIV infection (Arendorf and Holmes 2000; Holmes and Stephen, 2002; Robinson, 2002; Ranganathan and Hemalatha, 2006) as risk factors affecting the South African population.

A high prevalence of gingivitis and periodontitis was reported in African patients associated with poor nutrition and inadequate oral hygiene (Ranganathan and Hemalatha, 2006). A national health survey conducted in 1998 confirmed that oral diseases affect a large number of South Africans in older population groups, especially those considered non-urban Africans and people with little education (Naidoo *et al*., 2001). Gugushe (1998) studied the effect of race, education level and income on the prevalence of PD in the adult South African population and deduced that socio-economic variables appear to influence the frequency distribution of PD.

Worldwide studies have identified diabetes as a substantial risk factor associated with PD. Matu *et al.* (2009) confirmed that diabetics in a group of coloured and black communities in South Africa had a higher prevalence of PD than non-diabetics in the control group. A study conducted in a rural area in South Africa on type II diabetics found that PD was common (42%) in patients who had poorly controlled diabetes (Peck *et al.*, 2006). An estimated 2 million South Africans are living with diabetes (Butler, 2009), thus identifying diabetes as a serious risk factor in PD.

Oral lesions associated with HIV vary in developing countries compared to developed countries (Holmes and Stephen, 2002; Ranganathan and Hemalatha, 2006). PD in HIV infection is significant as "they may alert the presence of HIV infection or the progression of the disease and they may have relevance to dentists for service planning and the treatment of individual patients" (Robinson, 2002). There is a higher prevalence of HIV-associated PD in developing countries compared to developed countries (Arendorf and Holmes, 2000). In 2010, the number of South Africans living with HIV was estimated at 5.24 million (Statistics South Africa, 2010), therefore this is likely to be a significant factor affecting in the presence of PD in the South African population.

2.3. Current Treatment of Periodontal Disease

Mirth (1987) stated that "…accessibility of the oral cavity, the tolerance of the individuals to a variety of intra-oral appliances, and the known benefits of therapeutic agents in the prevention and treatment of oral disease, combined with the ability of compact controlled-release therapeutic systems to deliver medicinal agents at a predictable rate for extended periods, make the development of therapeutic systems for oral disease a logical outcome." This statement explains the initiative to develop a controlled release formulation for the treatment of PD. Several approaches to the treatment of PD exist. The choice of treatment is dependent on the type of PD present and the therapeutic objective of treatments as well as determining anatomical variations present and extent of attachment loss (Research, Science and Therapeutic Committee of the American Academy of Periodontology, 1997). Treatment of PD can be broadly categorised into, surgical and non-surgical treatments. The focus of this chapter is limited to nonsurgical treatments relating to pharmacological interventions.

The goal when treating PD is eradication of microorganisms followed by regeneration of structures destroyed by the disease (Research, Science and Therapeutic Committee of the American Academy of Periodontology, 2001). Scaling and root planing (SRP) forms the crux of periodontal therapy involving the removal of calculus and plaque (Pihlstrom *et al.*, 2005; Ryan, 2005). SRP combined with home care can lead to positive outcomes, but this may be hard to achieve (Chapple, 2009). Multiple clinical trials have proved SRP leads to a reduction

in microbial load, reductions in bleeding time upon probing and gain in attachment levels. However, this time-consuming process inevitably leaves behind microorganisms leading to recolonisation (Ryan, 2005). Pharmacological therapy is often used in combination with SRP delivering one or more chemotherapeutic agents. These may be delivered either systemically or locally within the periodontal pocket. Antimicrobial and anti-inflammatory agents have shown to be beneficial in the treatment of PD. These two broad categories of pharmacological agents used in the treatment of PD is discussed with the focus on their mechanism of action, particular drugs used, form in which the drug is delivered, and their efficacy in the treatment of PD.

2.3.1. Anti-inflammatory agents

Anti-inflammatory agents are classified in two broad categories based on their inhibition of the COX isoforms. COX-1 selective agents and COX-2 selective agents inhibit cyclooxygenase enzyme 1 and 2 respectively. Non-steroidal anti-inflammatory drugs (NSAIDs) non-selectively inhibit COX-1 and COX-2 enzymes, with various NSAIDs displaying different affinities for these enzymes.

NSAIDs have been investigated for their potential role in PD treatment. Jeffcoat *et al.* (1988) investigated the effect of flurbiprofen (50mg) administered twice daily for two months. Their investigation concluded that a significant decrease in bone loss was evident in the group receiving flurbiprofen compared to the placebo group. Vardar *et al.* (2003) compared oral administration of a relatively selective COX-2 inhibitor (nimesulide) and a non-selective COX-1 and COX-2 inhibitor (naproxen) on the gingival tissue levels of PG E_2 and PG $F_{2\alpha}$. Nimesulide inhibited PG $F_{2\alpha}$ sufficiently, however insignificant inhibition of PG E_2 was evident while naproxen inhibited PG E_2 significantly. Offenbacher *et al.* (1989) reported elevated levels of PG reaching a maximum at 6 months in animal models resulting in the adoption of the principle of using anti-inflammatory agents over an extended period of time for the treatment of PD. Although NSAIDs demonstrate inhibition of COX-1 and COX-2 enzymes, their systemic side effects may limit their long term application in PD leading to the use of COX-2 selective agents. Non-selective inhibition of the COX enzyme leads to interference of the production of PG E_2 and PG I_2 which stimulate the production of a cytoprotective mucous, without which leads to damage of the gastric mucosa (Salvi and Lang, 2005).

An over expression of COX-2 mRNA and protein levels are elevated in chronic periodontitis compared to healthy subjects (Zhang *et al*., 2003). This suggests a possible target for pharmacological treatment. In a double-blind study where 60mg Loxoprofen, a selective COX-2 inhibitor, in conjunction with SRP was administered to the experimental group, while the control group received SRP in conjunction with a placebo over the 28-day period (Pinho

Mde *et al.*, 2008). Both groups had successful reduction of inflammation and promoted pocket size reduction on day 28 of the study. The experimental group receiving loxoprofen demonstrated a slight benefit compared to the placebo group. Although COX-2 selective anti-inflammatories do not affect the gastrointestinal system, certain COX-2 selective anti-inflammatories have been associated with cardiovascular risks (Mukherjee *et al.*, 2001).

Another chemotherapeutic agent targeting the immune response pathway is low doses of doxycycline (20mg per capsule), commercially branded as Periostat®. The FDA approved the subantimicrobial dose of doxycycline, which is not sufficient to inhibit the growth of periodontal bacteria, however it reduces production of collagenase, the enzyme responsible for the destruction of gingival tissues. Thomas *et al.* (1998) conducted a 9-month study, researching the long-term subantimicrobial doses of doxycycline and confirmed that the administration of Periostat® was not associated with the emergence of tetracycline-resistant bacteria. Golub *et al.* (2001) studied the effect of subantimicrobial doses of doxycycline on collagenase activity and attachment loss in adult periodontitis conducting a three-part, placebo-controlled, double-blind, parallel group study. After completion of the 36-week study reduction of collagenase activity and improved levels of periodontal attachment were evident.

The emergence of targeted drug delivery systems lead to the development of a local drug delivery devices which are placed within the periodontal pocket loaded with an anti-inflammatory agent. Numerous devices, summarised in Table 2.3, have been formulated with various anti-inflammatories in an assortment of polymeric systems. The majority of these studies were limited to *in vitro* results only, however, clinical data is necessary in determining the success of locally delivering anti-inflammatories to the periodontal pocket.

Table 2.3: Local drug delivery devices containing anti-inflammatory agents

Polymer matrix	*Archetype*	*Model drug incorporated*	*Data available*	*Reference*
Atelocollagen	Film	Meloxicam	*In vitro*	Cetin *et al.*, 2005
Cellulose acetate	Film	Indomethacin / meloxicam	*In vitro*	Cetin *et al.*, 2004
2-ethylhexyl acrylate, 2-hydroxyethyl methacrylate & methacrylic acid	Film	Ketorolac	*In vitro*	Wong and Nguyen, 1998
Poly(L-lactide) (PLA)	Membrane cast on poly(glycolide) mesh	Flurbiprofen	*In vitro*	Park *et al.*, 1997
Poly(anhydride ester)	Membranes	Salicylic acid	*In vivo* (animals)	Erdmann and Uhrich, 2000

Systemic and local administration on a rat model of selective inhibitors of COX-1 and COX-2 agents were investigated to determine the role played by COX isoforms (Queiroz-Junior *et al.*, 2009). Indomethacin, celecoxib, SC 236 {4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide} and SC 560 [5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethyl pyrazole] were the anti-inflammatory agents used, injected by subcutaneous bolus injection when dosed systemically or injected into the gingival tissue at the buccal side when administered locally. The results of the study revealed the use of both selective and non-selective COX inhibitors reduce the signs of the disease in periodontal tissues as well as confirming that administration, either locally or systemically, of COX inhibitors would be effective. It was further shown that treatment with local administration of NSAIDs would reduce systemic side effects associated with these drugs.

Cetin *et al.* (2004) loaded cellulose acetate films with anti-inflammatory agent's indomethacin or meloxicam to study their *in vitro* release behaviour. Indomethacin released the quickest, at a rate of 0.022mg/hr, for the first 8 hours then dropping to 0.01mg/hr between 24 and 118 hours. A total of 70% of the entrapped drug was release over the time of the study. In comparison, meloxicam-loaded films released a total of 30% of drug over 118 hours at an initial rate of 0.8mg/hr for the first 8 hours then reducing substantially to 0.4mg/hr between 24 and 118 hours. The study was the first of its kind investigating the sustained release rate of an anti-inflammatory agent over extended period of time from a cellulose acetate film.

Park *et al.* (1997) proposed the development of a polymeric membrane consisting of a poly(glycolide) mesh which a poly(L-lactide) solution is spun onto. These membranes were to be placed subgingivally as part of guided tissue regeneration treatment. Flurbiprofen or

tetracycline was loaded into the membranes and were chosen for their effect on alveolar bone loss through inhibiting arachidonic acid metabolism and their anti-collagenolytic activity respectively. *In vitro* drug release profiles revealed constant release of either drug over 7 days; however tetracycline membranes initially demonstrated burst release. Drug release was dependent on hydrophobicity of the entrapped drug as well as the porosity of the membranes. The *in vitro* studies demonstrated the potential of the biodegradable membrane as a tool in improving guided regeneration techniques.

2.3.2. Antimicrobial agents

Eradication of pathogens from the periodontal cavity is the goal of antimicrobial treatment (Goodson, 1994). Antimicrobials may be delivered locally within the periodontal pocket or administered systemically.

Systemic antibiotic therapy is not routinely recommended for patients diagnosed with gingivitis or periodontitis, but rather restricted to patients who continue to lose attachment while receiving SRP (Research, Science and Therapeutic Committee of the American Academy of Periodontology, 2004). Table 2.4 summarises the antibiotics commonly used in the treatment of PD including their mechanism of action as well as their activity against periodontal pathogens.

A study conducted by Haffajee *et al.* (1995) compared clinical and microbiological outcomes in four groups each receiving a systemically administered agent namely, Augmentin®, tetracycline, ibuprofen and a placebo for 30 days. The data revealed that on average all groups achieved an overall gain in periodontal attachment. However, the Augmentin® and tetracycline group at 10 months showed more periodontal attachment compared to the ibuprofen and placebo groups. Furthermore, a reduction in periodontal pathogens 10 months post therapy was evident especially in the antibiotic groups yet no pathogen was completely eradicated.

Resistance of pathogens to antimicrobials is a rising problem. Ardila *et al.* (2010) studied the susceptibility of *Aggregatibacter actinomycetemcomitans* and *P. gingivalis* to systemically delivered antimicrobials; clindamycin, metronidazole, amoxicillin and moxifloxacin and amoxicillin/clavulanic acid. The results revealed that both pathogens demonstrated resistance to amoxicillin, clindamycin and metronidazole while all isolates of these two pathogens were susceptible to amoxicillin/clavulanic acid and moxifloxacin.

Numerous studies which have focused on the development of local drug delivery of antimicrobials to the supragingival and subgingival regions are summarised in Table 2.5. Antimicrobial agents contained within local devices is recommended for use as an adjunct to

SRP in chronic periodontitis with a pocket depth greater than or equal to 5mm that has not responded to conventional therapies with inflammation still present (Research, Science and Therapeutic Committee of the American Academy of Periodontology, 2006). The four products which are mostly widely used and commericially available in either the USA or UK are expanded upon following Table 2.4.

Table 2.4: Mechanism of action of antimicrobial agents frequently used in the treatment of PD including sensitivity of periodontal pathogens to specific antimicrobials

	Mechanism of action	*Example of antimicrobial used in PD*	*Notable activity against certain periodontal pathogens*	*References*
Cell wall	*Penicillins* Inhibit peptidoglycan synthesis	Amoxicillin and Clavulanate	*A. actinomycetemcomitans* and *P. gingivalis*	Ardila *et al.,* 2010
Protein synthesis	*Lincosamides* Interfere with initiation complexes and translocation reactions on the 50S ribosomal subunit	Clindamycin	Spirochetes, motile rods, *Prevotella intermedia*, *P. gingivalis*, *Selenomonas* spp., *Campylobacter rectus*	Sauvêtre *et al.*, 1993
	Macrolides Reversibly binding to the 50S ribosomal subunit	Azithromycin,	Spirochetes Gram-negative anaerobes especially *Fusobacterium* spp., *Bacteroides* spp., *Wolinella* spp. , *A. actinomycetemcomitans*, *Selenomonas* spp., *Mitsuokella multiacida*	Sefton *et al.,* 1996 Williams *et al.,* 1992
		Clarithromycin	Gram-positive anaerobes including *Actinomyces* spp. , *Propionibacterium* spp. , *Lactobacillus* spp., *Bifidobacterium dentium*	Williams *et al.*, 1992
	Tetracyclines Reversibly bind to the 30S ribosomal subunit	Doxycycline	*A. actinomycetemcomitans* and *P. gingivalis* *Serratia marcescens*, *Enterobacter cloacae*, some strains of *Enterobacter sakazakii*, *Acinetobacter baumannii*	Lavda *et al.,* 2004 Barbosa *et al.*, 2001
		Tetracycline	*Fusobacterium nucleatum*, *P. gingivali*, *Prevotella intermedia*, *Eikenella corrodens*, *Campylobacter rectus*, *A. actinomycetemcomitans*	Lowenguth and Greenstein, 1995
DNA	*Fluoroquinolones* Inhibit one or more of a group of enzymes called topoisomerases that are essential for bacterial DNA replication and transcription; inhibit DNA gyrase	Ciprofloxacin	*A. actinomycetemcomitans* *Pseudomonas aeruginosa, Serratia marcescens, Klebsiella oxytoca, Enterobacter cloacae, Enterobacter sakazakii, Kluyvera cryocrescens, Acinebacter baumanni*	Conway *et al.* 2000; Lavda *et al.* 2004 Barbosa *et al.*, 2001
	Nitroimidazole Interfere with nucleic acid synthesis resulting in the loss in DNA structure, strand breakage and impaired DNA function.	Metronidazole	Strong activity against Gram-negative anaerobes particularly spirochetes, *Selenomonas* spp., motile robs and *Prevotella intermedia*	Loesche *et al.* 1992

Table 2.5: Local drug delivery devices containing antimicrobial agents

Polymer matrix	*Archetype*	*Model drug incorporated*	*Data available*	*Reference*
Formulations containing one polymer:				
Atelocollagen	Film	Tetracycline	*In vivo* (humans)	Minabe *et al*., 1989
Cellulose acetate	Fibre	Tetracycline	*In vivo* (humans)	Goodson *et al*., 1979
Chitosan	Film	Ciprofloxacin	*In vitro*	Ahmed *et al*., 2009
	Microspheres	Tetracycline	*In vitro*	Govender *et al*., 2005
Ethylcellulose	Film	Minocycline	*In vivo* (humans)	Elkayam *et al*., 1988
	Film	Ornidazole	*In vitro*	Ramachandra *et al*., 2011
Ethyl vinyl acetate	Fibre	Tetracycline	*In vivo* (humans) Commercially available product Actisite®	Goodson *et al*., 1991
Hydroxypropyl cellulose	Strip	Tetracycline	*In vivo* (humans)	Noguchi *et al*., 1984
	Strip	Doxycycline	*In vivo* (humans)	Taner *et al*., 1994
Hydoxypropyl methylcellulose	Ointment	Minocycline	*In vivo* (humans)	Nakagawa *et al*., 1991
	Strip	Doxycycline	*In vivo* (humans)	Taner *et al*., 1994
Methylcellulose	Strip	Doxycycline	*In vivo* (humans)	Taner *et al*., 1994
Poly(ε-caprolactone) (PCL)	Film	Minocycline	*In vitro*	Kyun *et al*., 1990
	Fibres	Gentamycin	*In vitro*	Chang *et al*., 2008
	Nanofibres	Metronidazole	*In vitro*	Zamani *et al*., 2010
Poly-co-glycolic acid (PLGA)	Strip	Tetracycline / metronidazole	*In vivo* (humans)	Deasy *et al*., 1989
	Film	Amoxicillin & metronidazole	*In vitro*	Ahuja *et al*., 2006
	Strip	Tetracycline	*In vivo* (humans)	Maze *et al*., 1995
	Film	Tetracycline	*In vivo* (humans)	Agarwal *et al*., 1993
	Gel	Tetracycline	*In vivo* (humans)	Maze *et al*., 1996
	Microparticles	Tetracycline	*In vitro*	Esposito *et al*., 1997
	Microparticles	Minocycline	*In vivo* (humans) commercially available Arestin®	Van Dyke *et al*., 2002 Paquette, 2002
	Film	Metronidazole	*In vivo* (humans)	Golomb *et al*., 1984
	Film	Tetracycline	*In vitro*	Agarwal *et al*., 1993
Polyethylmetha acrylate (acrylic)	Strip	Tetracycline / metronidazole	*In vivo* (humans)	Addy *et al*., 1988 Addy and Langeroudi, 1984
Polyhydroxybutyric acid	Strip	Tetracycline / metronidazole	*In vivo* (humans)	Deasy *et al*., 1989
PLA	Membrane cast on Poly(glycolide) mesh	Tetracycline	*In vitro*	Park *et al*., 1997
Poly(ortho esters)	Semi-solid	Tetracycline	*In vivo* (humans)	Roskos *et al*., 1995 Schwach-Abdellaoui *et al*., 2002

Polymer matrix	*Archetype*	*Model drug incorporated*	*Data available*	*Reference*
Formulations consisting of two or more polymers:				
Chitosan & PCL	Film	Metronidazole	*In vivo* (humans)	El-Kamel *et al*., 2007
Ethyl cellulose & Polyethylene glycol	Film Patch	Tetracycline Tetrcycline / Carvacrol	*In vitro* *In vitro*	Azoury *et al*., 1998 Obaidat *et al*., 2011
Eudragit L® & Eudragit S®	Film	Clindamycin	*In vivo* (animals)	Higashi *et al*., 1991
Glycerol monooleate & sesame oil	Gel	Metronidazole	*In vivo* (humans) commercially available - Elyzol®	Noyan *et al*., 1997
Hydroxyethyl cellulose & polycarbophil	Gel	Metronidazole	*In vitro*	Jones *et al*., 1997
Hydroxyethyl cellulose & polyvinylpyrrolidone	Gel	Tetracycline	*In vitro*	Jones *et al*., 1996
Hydroxypropyl cellulose & carbomer 940	Tablet	Metronidazole	*In vitro*	Perioli *et al*., 2004
Hydroxypropyl cellulose, hydroxyethyl cellulose, Eudragit RL-100 & ethylcellulose	Strip	Sparfloxacin	*In vitro*	Muchalambe *et al*., 2010
Hydroxypropyl cellulose & methacrylic acid	Strip	Ofloxacin	*In vivo* (humans)	Higashi *et al*., 1990
Hydroxypropyl methylcellulose & sodium alginate	Gel	Doxycycline	*In vitro*	Obaidat *et al*., 2010
Polyhydroxybutyric acid & PLA	Compact	Tetracycline	*In vivo* (humans)	Collins *et al*., 1989
PLGA & methoxypoly(ethylen eglycol) / diblock copolymer [poly(D,L-lactic acid)-block-methoxypolyethylene glycol)]	Film	Tetrcycline hydrochloride	*In vitro*	Owen *et al*., 2010
Poly($_{DL}$-lactide) & N-methyl 2-pyrrolidone	Gel	Doxycycline	*In vivo* (humans Commercially available - Atridox®	Polson *et al*., 1997
PLGA & PCL	Microparticles	Doxycycline	*In vivo* (humans)	Mundargi *et al*., 2007
Polyvinyl alcohol & carboxymethyl chitosan	Film	Ornidazole	*In vivo* (animals)	Wang *et al*., 2007
Poly(vinyl methyl ether-co-maleic anhydride) & poly(ethylene oxide)	Film	Metronidazole	*In vitro*	Li and Lee, 2010

The first locally acting drug delivery device to be FDA approved comprised of tetracycline hydrochloride embedded in an ethylene vinyl acetate fibre, marketed as Actisite® (Actisite Pharmaceuticals Inc., North Andover, Massachusetts). The fibre, 23cm long and a of 0.5cm diameter, is placed within the periodontal pocket and removed after 10 days. Tonetti *et al.* (1990) reported a zero-order release of tetracycline over a 10-day period and the device maintained a constant average concentration of 1590µg/mL in periodontal pockets. In one study locally delivered tetracycline produced similar clinical outcomes to amoxicillin/clavulanic acid delivered systemically after SRP treatment over the 9-month observation period (Purucker *et al.*, 2001).

Atridox®, manufactured by Tolmar Inc. (Fort Collins, Colorado) contains 42.5mg of doxycycline hyclate within a liquid polymeric system comprising of poly($_{DL}$-lactide) & *N*-methyl 2-pyrrolidone. The product is packaged as a two-syringe mixing system, that once administered it flows to the bottom of the periodontal pocket and solidifies, releasing doxycycline over a 7-day period. Research conducted in two multi-centre studies with 411 patients in each group with severe periodontitis, found doxycycline delivered from Atridox was equally effective as SRP over a 9 month period. Salvi *et al.* (2002) designed a single-blind, randomised, parallel-designed clinical trial comparing Atridox®, Elyzol® and PerioChip®. The outcome of the trial was a recommendation that Atridox® to be used in conjunction with SRP in periodontal pockets with a residual depth of at least 6mm.

Microparticulate minocycline system, Arestin® (OraPharma Inc., Warminister, Pensylvania), comprises of PLGA based microspheres which are injected directly into the periodontal pocket. Arestin® releases minocycline at a constant rate of 340µg/mL in crevicular fluid over a 14-day period (Williams *et al.*, 2001). *In vivo* studies confirmed that minocycline in conjunction with SRP is more effective in reducing pocket depth than SRP alone (Williams *et al.*, 2001; van Dyke *et al.*, 2002, Paquette, 2002).

Colgate Elyzol® 25%$^{w}/_{w}$ Dental Gel (Colgate-Palmolive (UK) Limited, Surrey, UK) delivers metronidazole directly into the gingival crevice. The glycerol monooleate and sesame oil matrix has a low melting point that increases in viscosity within the periodontal pocket. After one application of the gel, metronidazole levels for 24 hours are maintained above the minimum inhibitory concentration required to inhibit growth of 50% (MIC_{50}) of the susceptible pathogens (Stoltze, 1992). Griffiths *et al.* (2000) conducted a blind, randomised split-mouth designed study revealing that metronidazole in combination with SRP is superior to SRP alone in the treatment of chronic adult periodontitis over a 9-month period.

2.3.3. Antiseptic agents

The role of chlorhexidine in PD, a cationic biguanide bactericidal antiseptic, has been extensively studied. This positively charged molecule attaches to the negatively charged bacterial cell wall and disrupts the osmotic balance. Its antibacterial spectrum includes Gram-positive bacteria, yeasts and fungi while it is less active against Gram-negative bacteria (Puig Silla *et al.*, 2008). As chlorhexidine was the first antimicrobial shown to inhibit plaque formation and prevention of gingivitis when a 0.2% mouthrinse was used daily, it provided a benchmark against which other antiplaque agents are compared (Addy, 1986). Still today chlorhexidine mouthwashes are frequently used in the prevention and treatment of gingivitis (Barnett, 2003; van Zyl and van Heerden, 2010). However, subgingival plaque is not significantly disrupted when chlorhexidine is applied supragingivally (Radford *et al.*, 1992), therefore limiting its use in PD (van Zyl and van Heerden, 2010). Cosyn *et al.* (2006) completed a randomised, controlled, single-blind, parallel study in which the chlorhexidine was applied as a varnish following SRP in 12 patients with chronic periodontitis. The pilot study revealed a reduction in pocket depths (between 0.7 - 1.37mm) and a gain in clinical attachment proving its potential in PD treatment.

Chlorhexidine is a popular drug of choice loaded into a device for implantation in the periodontal pocket (Table 2.6). It was incorporated into a crosslinked hydrolysed gelatin and glutaraldehyde film containing 2.5mg of drug and is commercially available as Periochip® (Dexcel Pharma Technologies Ltd., Jerusalem, Israel). The film releases 40% drug within 24 hours and the remainder over 7-10 days (Divya and Nandakumar, 2006). Studies revealed that Periochip® used in conjunction with SRP significantly improves clinical parameters of periodontitis (Killoy, 1999; Heasman *et al.*, 2001). When compared to other commercially available products Atridox® and Elyzol®, Periochip® performed moderately (Salvi *et al.*, 2002). This study further reported an increase in pocket probing depth 4 months after application, which may be attributed to the wafer dimensions.

Table 2.6: Local drug delivery devices loaded with chlorhexidine

Polymer matrix	*Archetype*	*Model drug incorporated*	*Data available*	*Reference*
Cellulose acetate	Film	Chlorhexidine	*In vitro*	Cetin *et al*., 2004
Chitosan	Film/gel	Chlorhexidine	*In vitro*	İkinci *et al*., 2002
	Chip	Chlorhexidine	*In vivo* (humans)	Jothi *et al*., 2009
Ethyl cellulose	Film	Chlorhexidine	*In vitro*	Friedman and Golomb, 1982
Ethylene vinyl acetate copolymer	Film	Chlorhexidine	*In vitro*	Tallury *et al*., 2007
	Film	Chlorhexidine	*In vitro*	Arnold *et al*., 2008
Gelatin	Film	Chlorhexidine	*In vitro*	Steinberg *et al*., 1990
	Film	Chlorhexidine	*In vivo* Commercially available as Periochip ®	Heasman *et al*., 2001
Hydroxyethyl methacrylate & polyethyleneglycol dimethacrylate	Gel plus nanocomposites	Chlorhexidine	*In vitro*	Bako *et al*., 2008
Hydroxypropyl cellulose	Strip	Chlorhexidine	*In vivo* (humans)	Noguchi *et al*., 1984
Polyethylmetha acrylate (acrylic)	Strip	Chlorhexidine	*In vivo* (humans)	Addy *et al*., 1988 Addy and Langeroudi, 1984
PLGA	Microspheres compressed into a chip	Chlorhexidine	*In vitro*	Yue *et al*., 2004
Polyurethane	Film	Chorhexidine	*In vitro*	Huynh *et al*., 2010
Urethane dimethacrylate & triethyene dimethacrylate	Disks	Chlorhexidine	*In vitro*	Anusavice *et al*., 2006

2.3.4. Combination therapies: Anti-inflammatory and antimicrobials agents

PD forms a complex set of diseases that are difficult to treat (Goodson, 1994). Hence, the rationale for the concomitant administration of an antimicrobial and an anti-inflammatory agent. This therapeutic approach, where controlled release of antimicrobials combined with anti-inflammatory agents, would control the periodontal disease and stimulate bone regeneration (Soskolne, 1997). The antimicrobial and anti-inflammatory agents would target the bacteria and inflammatory mediators simultaneously within the pocket.

To date only a few formulations (Table 2.7) simultaneously deliver an antimicrobial and anti-inflammatory agent. As yet no clinical data is available, although the *in vitro* results demonstrate promising potential in treating PD.

Table 2.7: Local drug delivery devices containing two or more drugs

Polymer matrix	*Archetype*	*Model drug incorporated*	*Data available*	*Reference*
Chitosan	Film	Ciprofloxacin & diclofenac	*In vitro*	Ahmed *et al*., 2009
Poly[1,6-bis(o-carboxyphenoxy) hexanoate]	Disk	Salicyclic acid & clindamycin / chlorhexidine / minocycline	*In vitro*	Johnson and Uhrich 2009

Ahmed *et al.* (2009) developed a chitosan-based ciprofloxacin and diclofenac film crosslinked with gluteraldehyde, which simultaneously delivers these two chemotherapeutic agents. The flexible films demonstrated *in vitro* drug release data with an initial burst release followed by controlled release over 7 days for the uncrosslinked film and 21 days for the crosslinked film. The films inhibited the growth of *Streptococcus mutans in vitro*. These results demonstrate the potential of the device in PD treatment.

A further *in vitro* study incorporating an antimicrobial agent and anti-inflammatory agent was conducted by Johnson and Uhrich (2009). An antimicrobial agent, chlorhexidine, clindamycin or minocycline, was incorporated into a salicylic acid-based polyanhydride-ester polymer blend. The resultant discs were *in vitro* evaluated and demonstrated no change in salicylic acid release when incorporated with the antimicrobial agents. Zero-order release of salicylic acid was achieved following a 15 hour lag period until reaching a plateau. The release profiles of the antimicrobials reflected the hydrophobicity of the drug influenced by its pKa. These discs demonstrated potential as a local device releasing the therapeutic agents for a minimum of three days.

2.3.5. Alternative chemotherapeutic therapies

Bisphosphonates inhibit bone resorption and show potential as host modulating therapies in the treatment of PD (Kirkwood *et al*., 2007). In a randomised clinical trial patients received one of two bisphosphonates or a placebo. The bisphosphonates groups demonstrated significantly improved clinical attachment levels, probing depth and bleeding on probing when compared to the placebo group at the 6- and 12-month intervals (Lane *et al.*, 2005). A controlled release formulation with alendronate sodium in PLGA microspheres tested *in vitro*, had a high drug-loading efficacy and demonstrated potential in dental applications (Nafea *et al*., 2007). Avascular bone necrosis, a side effect of bisphosphonates, may limit its dental applications (Robinson, 2004).

2.4. Design of Controlled Release Formulations for the Treatment of Periodontal Diseases

Since the 1970's scientists have been developing prolonged delivery devices for placement within the periodontal pocket for the treatment of PD. Numerous antimicrobials and anti-inflammatory drugs have been incorporated within a polymeric matrix which is secured within the periodontal pocket and the model drug is released over a extended period of time. The design of the devices can be broadly classified as fibres, films, gels or multiparticulate systems (Figure 2.1).

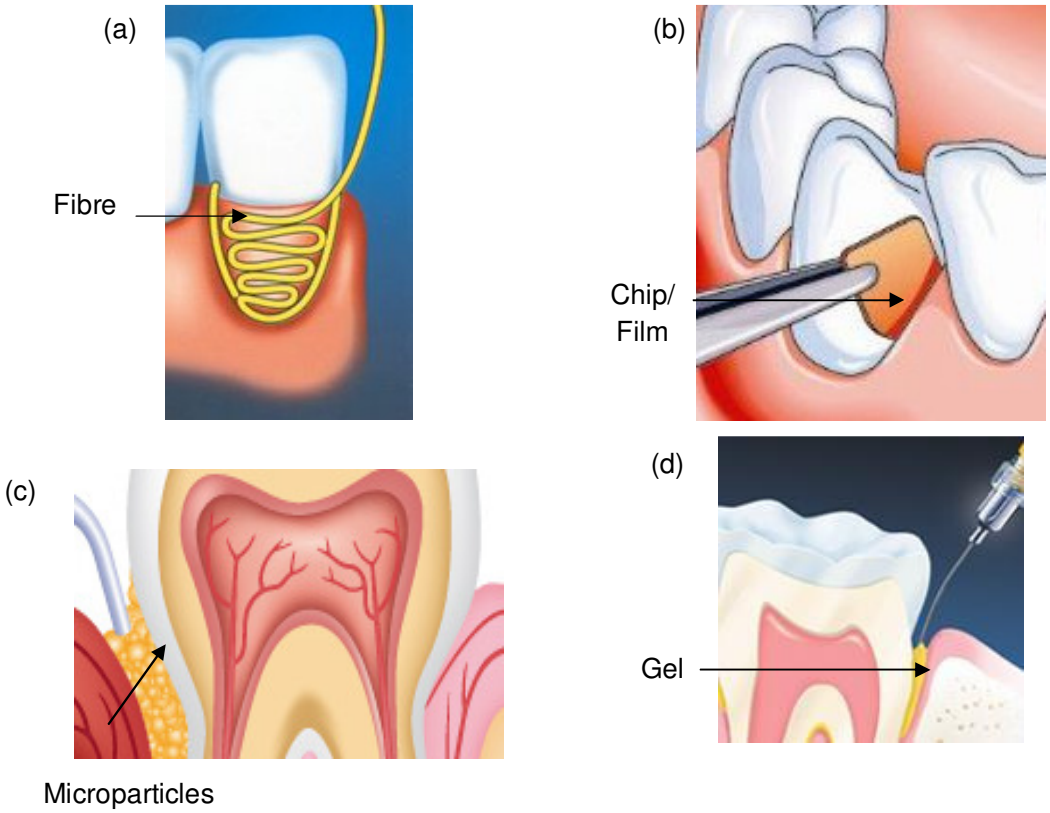


Figure 1.2: Representations of local drug delivery devices in the form of (a) a fibre (Adapted from Spiller, 2000), (b) a chip (Adapted from Hutter, 2008), (c) microparticles (Adapted from Harrington, 2011) and (d) a gel (Adapted from Tolmar Inc., 2008-2010)

2.4.1. Fibres

The first fibre for delivery of an antimicrobial agent was developed by Goodson *et al.*, (1979), where a hollow cellulose acetate fibre was filled with tetracycline, releasing 95% of the entrapped drug within 2 hours. Rapid release of the drug from a hollow fibre limited its clinical application. Subsequent development in fibre technology lead to the FDA approval of ethylene vinyl acetate fibre loaded with 12.3mg of tetracycline. This monolithic fibre releases tetracycline over 10 days. Fibres may either be place circumferentially into the pocket (Jain *et al.*, 2008) or overlapping on itself until the pocket is filled (Donley, 1997) as illustrated in Figure 2.1a. The fibre is then secured in place with a cyanoacylate adhesive. Difficulties in fibre placement and necessity to remove the non-degradable device have been identified as disadvantages of Actisite®.

2.4.2. Film/Chip

Films have been used more extensively as a possible device in the development of a local drug delivery system for the treatment of PD, as evident in the number of films which have been prepared and tested as decribed in Tables 2.3 and 2.5-2.7. Films may vary in thickness and size for optimal placement in the periodontal pocket. In recent years, films intended for application in PD use biodegradable and bioadhesive polymers. As a result the films do not have to be removed once treatment is complete and no longer require the additional adhesives to remain in the pocket; an example of such a film is Periochip®. A disadvantage if using a film is the difficulty securing the device in place with the normal flushing of the periodontal pocket (Jones *et al.*, 1996). It is hypothesised that another drawback of using a film compared to other devices is that the device is not evenly distributed in the pocket and the drug released is concentrated at the site of application.

2.4.3. Multiparticulate systems

Multiparticulates refer to both micro- and nanoparticulates which may be in the form of spheres, tubules, capsules, fibres or rods. These formulations optimise the theories of micrometrics, by using smaller particulates to alter the pharmacokinetic profiles of the polymeric matrices. Several studies have used the microparticles in the development of extended release formulations for the treatment of PD. Such formulations uses particle size technology to optimise drug delivery, either administered on its own (Gopinath *et al.*, 2009), compressed into a film or strips (Yue *et al.*, 2004) or suspended within a formulation such as a gel or paste (Mundargi *et al.*, 2007). Particles on the nanoscale affect the physicochemical and physicomechanical properties of the material such as melting point, electrical properties and optical properties (Kong *et al.*, 2006). However, few studies thus far have employed the use of nanoparticles as a potential carrier of therapeutic agents to the periodontal pocket

(Piñón-Segundo *et al.*, 2005; Moulari *et al.*, 2006; Shahverdi *et al.*, 2007; Satishkumar and Vertegel, 2008). Yet this relatively new technology demonstrates promising benefits for reaching deep within the periodontal pockets below the gum line, which is not possible with other devices. Nanoparticles are further formulated with biocompatible, biodegradable and bioadhesive polymers which add to their attractiveness as a delivery device. A problem noted by Piñón-Segundo *et al.* (2005) was that due to the increased surface area of the nanoparticles available for dissolution, drug release was faster than expected.

2.4.4. Gels

Several gels have been formulated as possible treatments for PD. Gels have a semi-solid consistency and therefore have the advantages of even distribution throughout the periodontal pocket. The gel comes into direct contact with the bottom of pockets and has faster drug release rates. A gel cannot be removed from the pocket following administration and for this reason biodegradable and biocompatible polymers are used, ensuring less irritation and allergic reactions. Together with bioadhesive polymers the gel is secured in the pocket. The key disadvantage of such systems is maintaining the formulation over an extended period of time within the pocket and the possibility of drug "dumping". A novel approach to overcome this problem was used in Atridox®, where the two part gel system solidifies within the periodontal. Solidification of the gel system in periodontal pocket results in controlled doxycycline levels over 46.73µg/mL in the ginigival crevicular fluid for 10 days (Kim *et al.*, 2004). To sustain higher drug levels over longer time frames multiparticulates, such as micro- and nanopaticles, may be suspended within the gel altering the drug release profile of the system.

2.5. Polymers Used in the Formulation of Controlled Release Formulations for the Treatment of Periodontal Diseases

Controlled release formulations consist of one or more drugs incorporated in a matrix of polymeric materials. Drug release from a controlled delivery device occurs through erosion, diffusion, osmosis and disintegration mechanisms and is dependent on three factors; drug solubility, polymer used and the drug loading (Tangri and Khurana, 2011). The chosen polymer incorporated has a pivotal effect on the release characteristics of the device. Controlled drug delivery devices can be classified as reservoir devices, where the device is removed and drug release is controlled by diffusion through a membrane, or as matrix devices, where the device degrades over time within the periodontal pocket releasing drug via diffusion or erosion (Banker and Rhodes, 2002). Although it is easier to control the time which the pocket is exposed to the therapeutic agent when using a non-degradable device, they have largely been replaced by biodegradable devices. This is because degradable

devices are more compatible and evoke less of an immune response as well as having improved patient compliance when compared to non-degradable ones (Vyas *et al*., 2000). Recently, the use of mucoadhesive polymers in the formulation of local delivery devices for the treatment of PD has increased. Incorporation of mucoadhesive polymers allows direct contact with the adsorbing membranes involving ionic interactions between the polymer and the mucous membrane (Bernkop-Schnürch, 2005). Polymers which have been commonly used in the formulation of local delivery devices intended for the treatment of PD are listed in Table 2.8.

Table 2.8: Polymers frequently used in the formulation of drug delivery devices placed within the periodontal pocket

Polymer	*Non-degradable*	*Biodegradable*	*Bioadhesive*	*Sustained release*	*Other*	*Reference*
Cellulose derivatives:						
Cellulose acetate		•				Goodson *et al.*, 1979; Cetin *et al.*, 2004
Ethyl cellulose	•			•		Golomb *et al.*, 1984
Hydroxyethyl cellulose		•	•	•		Jones *et al.*, 1996
Hydroxypropyl cellulose		•				Noguchi *et al.*, 1988
Hydroxypropyl methylcellulose		•	•	•		Perioli *et al.*, 2007
Chitosan		•	•		Antimicrobial	İkinci *et al.*, 2002
Carboxymethyl chitosan		•	•			Wang *et al.*, 2007
Ethyl vinyl acetate	•			•		Goodson *et al.*, 1983
Gelatin		•		Crosslinked		Cetin *et al.*, 2005
Hydroxyethyl methacrylate		•				Bako *et al.*, 2008
Polyacrylic derivatives:						
Carbopol 971		•	•			Perioli *et al.*, 2004; Perioli *et al.*, 2007
Carbomer 940		•	•			
Polycarbophil		•	•			
Poly(anhydride esters)		•		•	pH responsive release	Erdmann and Uhrich, 2000
PCL		•		•		Chang *et al.*, 2008
PLGA		•		•		Agarwal *et al.*, 1993
Polyethylene glycol		•				Golumb *et al.*, 1984
Polyethyleneglycol dimethacylate		•				Bako *et al.*, 2008
Polyhydroxybutyric acid		•		•		Collins *et al.*, 1989
PLA		•		•		Park *et al.*, 1997
Poly(*ortho* ester)		•	•		pH sensitive release	Roskos *et al.*, 1995
Polyvinyl alcohol		•		•	High tensile strength	Wang *et al.*, 2007
Polyvinylpyrrolidone			•			Jones *et al.*, 1996

2.6. Concluding Remarks

PD is a known problem among the South African population and is compounded by the high prevalence of diabetes and HIV. These chronic conditions require patients to take medication on a daily basis. Therefore, a local drug delivery system controlling drug release to the periodontal pocket would be ideal in treating these patients for PD in comparison to systemically delivered therapies. This is because it would not require the additional self-administered treatment and would reduce non-compliance.To date no such devices are routinely used to treat patients with PD in South Africa. The direct delivery of the drug to the affected area, the possible improved patient compliance and the large percentage of South Africans affected by PD, consequently provide the motivation for this study.

The review of literature found that antimicrobials and anti-inflammatories play a role in the treatment of PD, both having been administered systemically and locally. Prescribing systemic therapeutic agents remains a popular route of drug administration, however research internationally has been focused on the development of devices for the local delivery of drugs in the treatment of PD. Several devices formulated have failed to meet their full potential in the clinical phase of research and development (Schwan-Abdellaoui *et al*., 2000) resulting in only a few devices being available commercially abroad. There is still a need to develop intra-pocket periodontal systems that are biodegradable, adhere to the tooth's surface and penetrate deep within the periodontal pockets (Jain *et al*., 2008). Only a few studies have incorporated both antimicrobial and anti-inflammatory agents and there is no clinical data to date. It is perhaps possible that this dual therapeutic approach is the way forward. The challenge that faces researchers is to design a formulation that delivers the therapeutic agent at a predetermined rate and over an extended period of time as well as being easy and convenient to use.

In comparison to the fibres already developed for treatment of PD, the PFD formulated in this study, simultaneously delivers an antimicrobial and anti-inflammatory agent to the periodontal pocket. The device is biodegradable and biocompatible, therefore, would not need to be removed. A further advantage of the PFD is that the fibre, comprising of a specific polymer and plasticiser combination, is resilient yet malleable for easy placement within the periodontal pocket.

CHAPTER 3

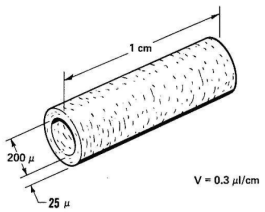
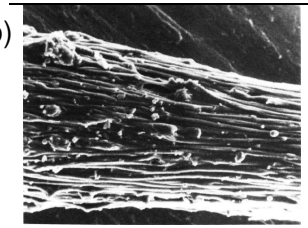
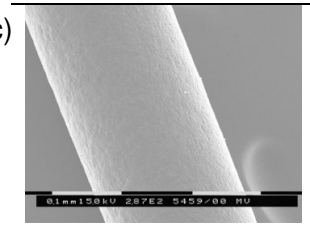
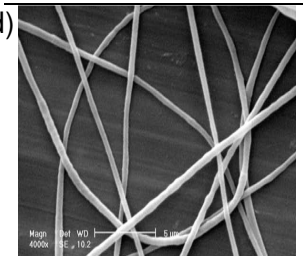
PRELIMINARY DESIGN OF A DRUG-LOADED POLYMERIC FIBRE DEVICE

3.1. Introduction

The review of formulations in Chapter 2, led to investigation into fibres as a delivery device for chemotherapeutic agents within the periodontal pocket. This chapter evaluates the fibres formulated specifically for the treatment of PD and attempts to design a formulation demonstrating potential as a device controlling the release of model drugs in the periodontal pocket. Few fibres have been formulated for the treatment of PD. Table 3.1 compares non-degradable fibres, composed of cellulose acetate and ethyl vinyl acetate, to biodegradable fibres prepared from PCL. Goodson *et al.* (1979) first published their results from loading tetracycline into cellulose acetate dialysis tubing (Table 3.1a). Once the hollow tube (outer diameter 250µm, inner diameter 200µm) was filled with tetracycline powder it was gently pressed into the periodontal pocket. The study showed that a dose reduction of over 1000 fold was achieved compared to systemic administration of the same drug. This was the first antimicrobial-loaded device formulated for the treatment of PD and provided a platform form which further fibre formulations could be developed.

Goodson *et al.* (1983) formulated a monolithic fibre where biocompatible polymers; polyethylene, polypropylene, PCL, polyurethane and cellulose acetate propionate, all released the drug *in vitro* within a day. However, ethylene vinyl acetate provided controlled delivery for up to 9 days (Table 3.1b). The ethylene vinyl acetate fibre demonstrated promising results *in vivo* maintaining constant tetracycline levels (1590µg/mL) over 10 days. The commercialisation of this tetracycline-loaded ethylene vinyl acetate fibre resulted in the product known as Actisite®. Although an improved clinical response is seen in these fibres administered in conjunction with SRP compared to SRP alone (Heijl *et al*., 1991, Newman *et al*., 1994), problems with the device have been identified. Fibre placement within the pocket is time consuming and requires trained personnel. Ethylene vinyl acetate fibres, a nondegradable device, need to be removed after treatment necessitating a further visit to the therapist. Furthermore, the fibres need to be secured within the pocket using cyanoacylate glue. These problems limit the use and acceptability of tetracycline-loaded ethylene vinyl acetate fibres (Soskolne, 1997; Vyas *et al*., 2000; Jain *et al*., 2008).

Table 3.1: Comparison of fibres formulated for the treatment of PD

	Image	*Characteristic*	*Dimensions*	*Reference*
(a)		Hollow tube Polymer: cellulose acetate Model drug: Tetracycline Drug release: 95% drug in 2 hours (in vivo humans)	Inner diameter: 200μm. Outer diameter: 250μm Tube: 25μm thick	Goodson *et al.*, 1979
(b)		Monolithic fibre Polymer: ethylene vinyl acetate Model drug: tetracycline Drug release: constant drug levels in pocket over 10 days (in vivo humans)	Diameter: 500μm	Tonetti *et al.*, 1990
(c)		Gravity spun fibre Polymer : PCL Model drug: Gentamicin Drug release: 10-50 days (in vitro)	Diameter range: 149-221μm	Chang *et al.*, 2008
(d)		Nanofibres Polymer: PCL Model drug: Metronidazole Drug release: 19 days (in vitro)	Diameter range: 313nm-999nm	Zamani *et al.*, 2010

Biodegradable fibre devices developed are gravity and electro spun which form a graft matrix-like structure which is placed within the periodontal pocket (Table 3.1c and d respectively). Thin fibres are formed resulting in an increased surface area for the diffusion of drug. PCL was the polymer incorporated in fibre formulations releasing gentamicin *in vitro* over 50 days from a nonhomogenised suspension of gravity spun fibres (Chang *et al*., 2008) and metronidazole over 19 days from electrospun nanofibres (Zamani *et al*., 2010). These novel approaches to fibre production produce a device which can more adequately be compared to a film than a fibre.

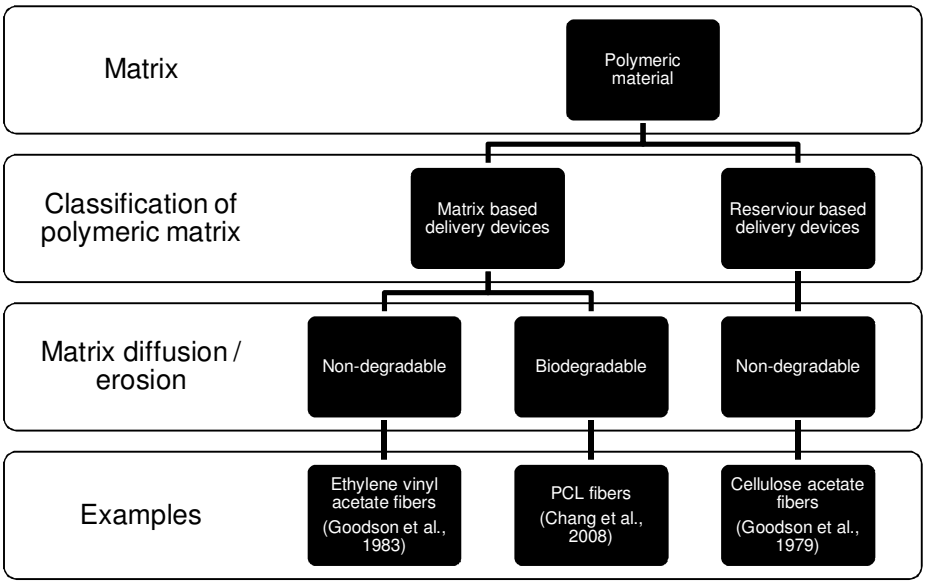


Figure 3.1: Classification of polymeric fibre matrices

Chapter 1 and Chapter 2 explained the rationale for local drug delivery of chemotherapeutic agents in the periodontal pocket as well as elucidating the use of antimicrobial and anti-inflammatory agents used in the treatment of PD. This chapter describes the design of a flexible and strong polymeric fibre intended to be placed within the periodontal pocket delivering the model drug over 10 days.

Alginate, which has been used extensively in wound care management, was crosslinked with a suitable cation forming the polymeric backbone of the fibre. Initially only ciprofloxacin was incorporated as a model antimicrobial drug for preliminary studies. Diclofenac sodium, the model anti-inflammatory agent, was incorporated in subsequent investigations. Alginate, a well known biocompatible polymer used extensively in the food and pharmaceutical industry, is a natural polysaccharide isolated from brown seaweed. The chemical structure of the "building blocks" of alginate are (1,4) linked α-L-guluronate and β-D mannuronate, will be referred to as the G and M monomers (Figure 3.2a and b correspondingly). These monomers

are joined together to form blocks and may arrange themselves in one of the following patterns (GM), (GG) and (MM). Arrangements of these monomers within these blocks determine the mechanical stability with increased G monomers resulting in high mechanical strength while higher M monomers lead to increased flexibility (Smidsrød and Skjåk-Bræk, 1990).

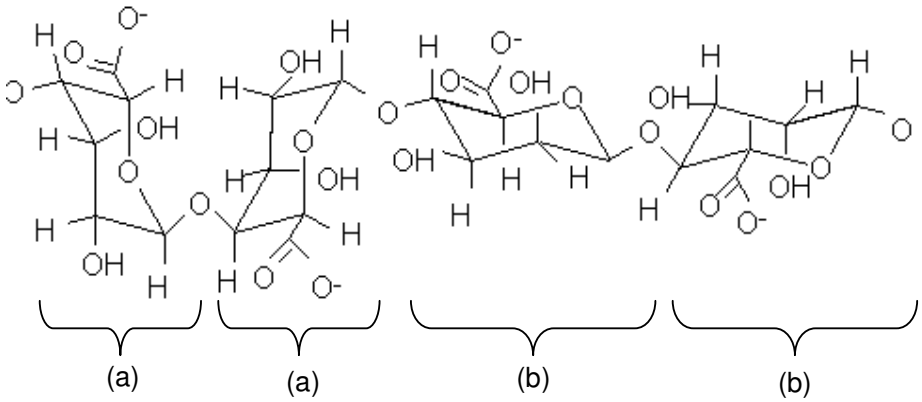


Figure 3.2: Monomers of alginate (a) (1,4) α-L-guluronate (G monomer) and (b) (1,4) β-D mannuronate (M monomer)

Multivalent cations bind to the carboxylic groups on the G monomers on two adjacent chains crosslinking the solution into a gel network (Augst *et al*., 2006). Haug and Smidrøds (1965) experimentally determined that the concentration of cations needed to induce gelation and precipitation of sodium alginate prepared from the *Laminaria digitata* and *Laminaria hyperborean* species in increasing order were:

$$Ba > Pb > Cu > Sr > Cd > Ca > Zn > Ni > Co > Mn,Fe > Mg$$

The “egg-box model” was developed by Grant *et al.* (1973) which describes the alignment of the polymeric chains into a two-dimensional model in the presence of cations resembling the shape of a corrugated egg box. This model, Figure 3.3, represents the alignment of the polymeric units forming a crosslinked structure resulting in a gelation of the solution. For the proposed fibre formulation barium chloride was selected as a suitable cation. Bajpai and Sharma (2004) determined that barium chloride is more stable in a medium of varying pH compared to calcium chloride and the large barium ion present in the polygluconate blocks maintained the integrity of the crosslinked beads investigated. Gels crosslinked with barium cations have a higher Young’s modulus than the calcium crosslinked gels attributed to a higher crosslinker density (Jejurikar *et al*., 2011), further justifying the incorporation of barium as the crosslinking cation.

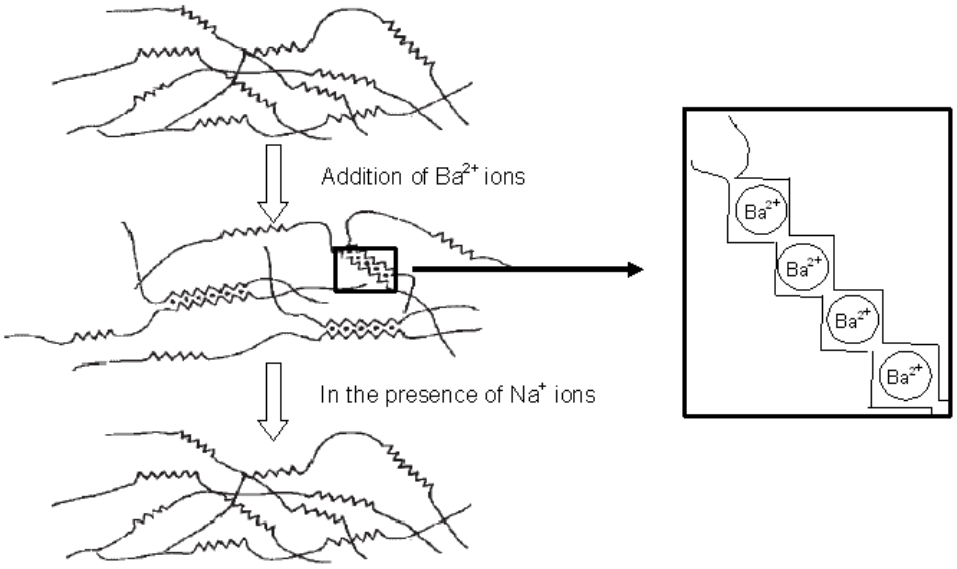


Figure 3.3: Alignment of alginate monomers upon addition of barium (Ba^{2+}) ions forming an "egg-box" structure which relaxes once again in the presence of sodium (Na^{+}) ions (Adapted from Qin, 2008)

3.2. Materials

Sodium alginate (Protanal LF 10/60) was purchased from FMC Biopolymer, Philadelphia, USA. Ciprofloxacin and metronidazole were sourced from Sigma-Aldrich, Steinheim, Germany. Barium chloride 2-hydrate was procured from Saarchem, Pty(Ltd), Unilab, Wadeville, South Africa. Glycerol (M_w=92.1g/mol) was purchased from Rochelle Chemicals, Johannesburg, South Africa. Dialysis tubing cellulose membrane, average flat width of 33mm, was acquired from Sigma-Aldrich, Steinheim, Germany. The antimicrobial susceptibility test disks (ciprofloxacin 1µg and metronidazole 50µg per disk) and Tryptone Soya Agar (TSA) were obtained from Oxoid, Hamphire and Hants, UK, respectively. Stock cultures of microorganisms were obtained from the National Health Laboratory Service. Buffering components; potassium dihydrogen orthophosphate, sodium hydroxide pellets and hydrochloric acid were correspondingly obtained from, Ace (Johannesburg), Merck (Wadeville) and Rochelle (Johannesburg).

3.3. Methods

3.3.1. Formulation of fibres

Initially alginate based polymeric fibres were prepared by adding a 3%$^{w}/_{v}$ alginate solution to 5%$^{w}/_{v}$ barium chloride solution, using a 10mL syringe fitted with an 18G needle, by applying constant pressure to the plunger. The formulation cured for 24 hours allowing the fibres to sufficiently crosslink. The resultant fibres were washed with deionised water and air dried for 48 hours at ambient conditions (25˚C). Additional components such as plasticisers and model drugs, depicted in Figure 3.4, were incorporated into the fibre formulation.

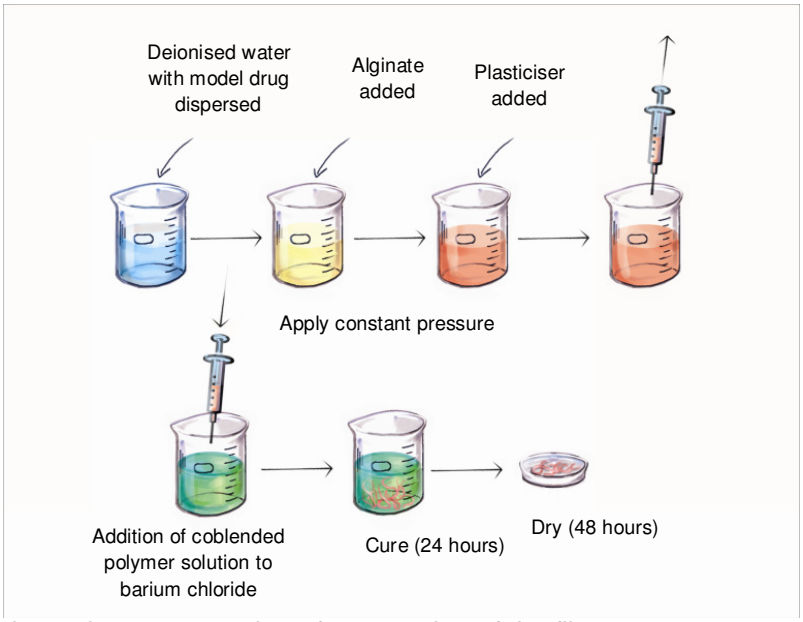


Figure 3.4: Schematic representation of preparation of the fibres

3.3.2. Characterisation of morphological transitions

Approximately 1cm fibres were secured on carbon tape, attached to sample holders and sputter coated (Spi-Module Sputter Coater, Apollo Scientific, Pennsylvania, USA) with gold for 90 seconds. Samples were then mounted in a scanning electron microscope (SEM) (Phenom Microscope, FEI Company, Hillsboro, Oregon, USA). The images produced compared the effects of formulation components on the morphological structure of the fibre.

3.3.3. Addition of suitable plasticiser

Fibres were prepared using the same method as in Section 3.3.1., however a 4%$^{w}/_{v}$ alginate solution crosslinked in a 2%$^{w}/_{v}$ barium chloride solution. Plasticiser (7-10mL) was added to the polymer solution prior to crosslinking. Plasticisers, triethanolamine or glycerol, were added to alginate solution to form a homogenous mixture. Table 3.2 indicates the plasticiser used and its concentration in each formulation.

Table 3.2: Fibre formulations incorporating either triethanolamine or glycerol at varying concentrations

Formulation	*Plasticiser*	*Plasticiser concentration*
A (control)	None	-
B	Triethanolamine	7mL
C	Triethanolamine	10mL
D	Glycerol	7mL
E	Glycerol	10mL

A *TA.XTplus* Texture Analyzer (Stable Micro Systems, England) was used for analysis of fibres. The tensile grip was calibrated at a 60mm distance from the sample stage. A fibre was secured to both the lower and upper custom made tensile grips, schematically represented in Figure 3.5. Table 3.3 below states the settings used in each test.

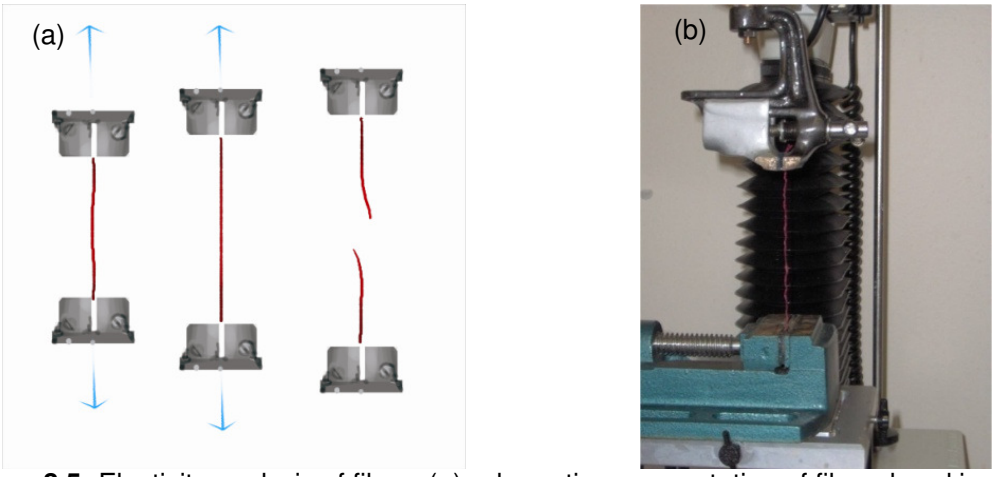


Figure 3.5: Elasticity analysis of fibres (a) schematic representation of fibres breaking when the maximum force is exceeded and (b) a fibre attached to a custom made clamp and upper gripper of the *TA.XTplus* Texture Analyzer

Table 3.3: *TA.XTplus* Texture Analyzer settings for elastic analysis of fibres

Settings	*Elasticity*
Mode	Measure force in tension
Option	Return to start
Pre-test speed	1.0mm/s
Test speed	3.0mm/s
Post-test speed	10.0mm/s
Distance	100mm
Trigger type	Auto – 15g
Data acquisition	400pps

3.3.4. Preparation of antimicrobial-loaded fibres and determination of drug entrapment efficiency

Ciprofloxacin and metronidazole were identified as possible antimicrobial agents to be incorporated within the crosslinked polymeric fibre. The fibres were prepared as described in Section 3.3.1. However, the antimicrobial drug (200mg), either ciprofloxacin or metronidazole, was dispersed in distilled water prior to the addition of alginate. Drug entrapment was determined through analysis of the drug remaining in the crosslinking solution once the fibres were removed. The amount of drug in the crosslinking solution was determined by ultraviolet spectroscopy (Specord 40, Analytik Jena, AG, Germany) at λ_{max}=278nm for ciprofloxacin or λ_{max}=275nm for metronidazole. Calibration curves were constructed in drug-free crosslinking solution individually for metronidazole and ciprofloxacin. All experiments were carried out in triplicate and then compared with standard calibration curves generated in crosslinking solution for each fibre. This quantity was deducted from the total amount of drug initially included for the formulation of the fibre. The difference between the two amounts is the quantity entrapped in the fibres as outlined in Equations 3.1 and 3.2.

$$Actual\ drug\ concentration = Drug\ added\ intially - Drug\ remaining\ in\ crosslinking\ solution \quad \textit{Equation 3.1}$$

$$DEE\ \% = \frac{Actual\ drug\ concentration}{Theorectical\ drug\ concentreation} \times 100 \quad \textit{Equation 3.2}$$

3.3.5. Determination of the antimicrobial activity of antimicrobial-loaded fibres

Agar plates were prepared with 100µL of culture and 20mL of molten TSA poured into sterile petri dishes and swirled to evenly distribute either *Escherichia coli* (*E. coli*) or *Enterococcus faecalis* (*E. faecalis*) cultures. *E. Faecalis* and *E.coli* are examples of Gram-positive and Gram-negative facultative anaerobes respectively, which have been found present in periodontitis (Colombo *et al*., 2002). Using a sterile pair of forceps, samples of fibres were transferred onto the solidified agar. Ciprofloxacin- or metronidazole-loaded fibres tested simultaneously with the drug-free fibres, which served as the control (Figure 3.6a). Control plates were prepared, using commercially available antibiotic-loaded disks (ciprofloxacin 1µg

and metronidazole 50µg) and were place on the solidified molten agar, which had been inoculated with the test pathogens. All the plates were placed in the fridge for 1 hour, allowing the drug to diffuse from the fibre or disc, after which they were incubated at 37˚C for 24 hours. Post incubation, plates were examined to determine the antimicrobial activity of the fibres through visualising the zone of inhibition. This is the area surrounding the fibre that is free from microbial growth, represented by the yellow area in Figure 3.6b.

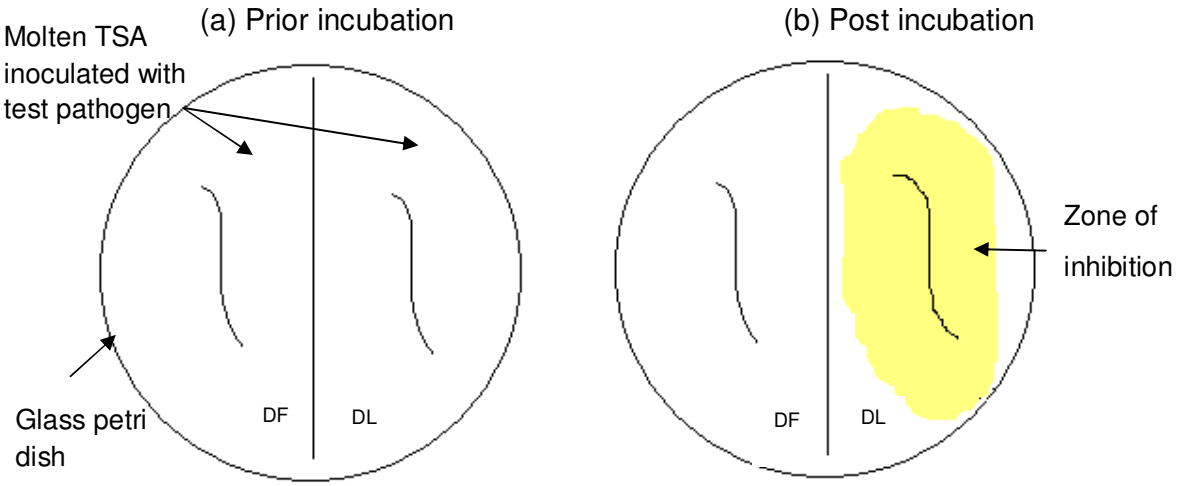


Figure 3.6: A diagrammatic sketch representative of agar diffusion plates with drug-loaded (DL) fibres tested alongside drug-free (DF) fibres (a) prior to incubation and (b) post incubation with the zone of inhibition represented by the yellow area

3.3.6. Determination of drug release from fibres

Dissolution studies were conducted in 0.2M phosphate buffer solution (PBS) at pH 4 and 6.8. PBS pH 4 and pH 6.8 were prepared according to the United States Pharmacopeia 23 (USP) (1995) recommendations. Simulated pockets were designed using cellulose membrane dialysis tubes, 3.3cm in diameter and 5cm in length. Ciprofloxacin-loaded fibres were weighed and placed within a simulated pocket. Either end was secured with a non-degradable nylon based thread. The simulated pocket was placed in 20mL PBS in a 50mL glass jar and left in a shaking incubator (50 rpm) (Orbital Shaker Incubator, LM-530, Lasec Scientific Equipment, Johannesburg, South Africa) for 10 days. A 2mL filtered (0.22µm syringe filter) dissolution sample was withdrawn at specific time intervals (0, 1, 2, 4, 6, 8 and 10 days) over the test period and sink conditions were maintained by replacing the withdrawn volume with PBS. The amount of drug released was determined by ultraviolet (UV) spectroscopy (Specord 40, Analytik Jena, AG, Germany) at λ_{max}=278nm for ciprofloxacin. The results were analysed using WinASPECT® Spectroanalytical Software (Analytik Jena AG, Jena). All experiments were carried out in triplicate.

3.3.7. Characterisation of nanoTensile properties

Mechanical properties of drug-free fibres were tested using creep-, tensile- and dynamic-loads. Tensile tests require prior determination of the cross-sectional area, length and test parameters. Cross-sectional area of the drug-free fibres was determined using stereomicroscope (Olympus SZX –ILLD2-200, Olympus Corporation, Tokyo, Japan). Samples for each were positioned using a custom-made clamp, exposing the cross-sectional area of the fibre. The tip of the fibre was coloured black using a permanent marker for adequate visualisation of the area. The area was calculated using the AnalySIS Olympus Soft Imaging Solutions version 5.1. The length was measured with digital callipers. The tensile analysis of drug-free fibres was determined using the nanoTensile™ 5000 (Hysitron Inc., Minneapolis, USA) (NT). Samples were mounted on metal brackets as outlined in Figure 3.7.

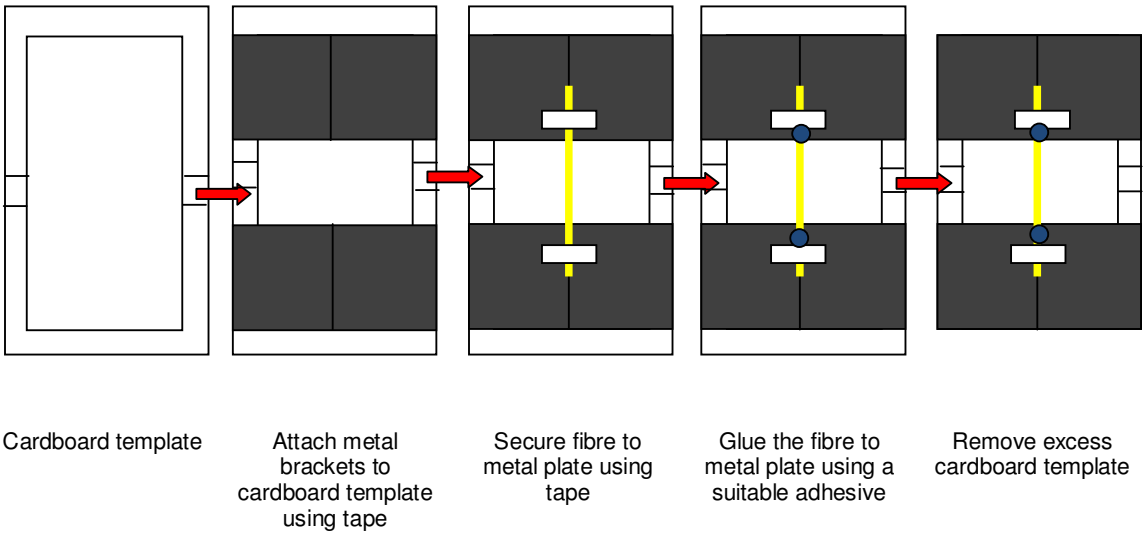


Figure 3.7: Schematic representation of fibres mounted on metal brackets secured on a cardboard template

The suitable test settings were chosen for either creep, tensile or dynamic-load analysis. Once the adhesive had dried, the samples were mounted on the upper gripper of the NT head and automatically weighed. The metal bracket was secured in place with tightening of the lower gripper and aligned. The cardboard segments between the two lines on either side of the template were then cut. Once the fibre had fractured or reached the time limit, the sample was removed from the upper and lower gripper. Plots generated were used to analyse the strength and flexibility of the fibre. The NT Head transducer constants were loaded as outlined in Table 3.4.

Table 3.4: Standard NT transducer settings

X, Y and Z NT Head transducer constants	*Setting*
X Force FP Gain	100
X Force FP Filter (Hz)	300
Y Force FP Gain	100
Y Force FP Filter (Hz)	300
Z Displacement FP Gain	100
Z Displacement FP Filter (Hz)	300
Z Force FP Gain	100
Z Force FP Filter (Hz)	300

3.3.7.1. Creep-load analysis

Creep-load analysis of fibres held either the load-limit or displacement-limit constant. For this study the load-limit was set at 2000mN and 2100mN for analysis of fibres. The applied stress is kept constant over the duration of the test. The test was conducted over 10 minutes or until the fibre fractured. The maximum strain, displacement and force were recorded either at the point of fracture or after 10 minutes if the fibre was still intact.

3.3.7.2. Dynamic-load analysis

Dynamic-load test exposes the fibre to a minimum and maximum load or displacement at a set frequency. The minimum and maximum load was set at 1000mN and 1500mN correspondingly, while the frequency was held at 0.01Hz.

3.3.7.3. Tensile-load analysis

The tensile-load may be controlled via by the displacement, load or strain rate through adjustment of the test velocity, load rate or strain rate respectively. The rate of velocity was regulated at 20µm/sec with a displacement-limit of 150mm and a load-limit of 1N. The data was analysed using the NT analysis software and revealed information pertaining to yield stress, ultimate strength, ultimate strain and toughness as well as calculating the Young's modulus for the fibres tested.

3.3.8. Effect of formulation components on formation of fibres

The effect of formulation components, polymer, crosslinking salt and plasticiser concentration, on fibre formation were observed. One factor at time was altered while the remainder factors kept constant. Fibres were prepared as outlined in 3.3.1 and observations regarding fibre formation were noted.

3.4. Results and Discussion

3.4.1. Fibre formulation

Fibres formed as the polymer solution made contact with the cations present in the crosslinking solution. The polymeric solution in the presence of barium ions forms a gel in the shape of a fibre due to the constant pressure applied to the plunger of the syringe. The alginate polymer used has a guluronic acid and mannuronic acid concentration of between 65-75% and 25-35% respectively. Analysis of the crosslinked fibres morphology (Figure 3.8) confirms that monolithic fibres were formed of a consistent shape and size (diameter range 393-458nm) with ridges evident on the surface of the fibres.

Figure 3.8: SEM images of 3%$^{w}/_{v}$ alginate fibres crosslinked in 5%$^{w}/_{v}$ barium chloride solution (a)-(d) illustrating the varying diameters (393-458µm)

3.4.2. Effect of plasticiser on elasticity of the fibre

The fibres formed without plasticiser (formulation A in Figure 3.9 and Table 3.5) break at lower breaking forces and extend less than their plasticised counter parts (formulations B-E in Figure 3.9 and Table 3.5) The incorporation of a plasticiser in the crosslinked alginate matrix improves the strength as well as the flexibility of the fibres. The volume and type of plasticiser added affected the tensile properties of fibres. When 10mL of plasticiser was incorporated (formulation C and E in Figure 3.9 and Table 3.5) weaker fibres were produced that fractured at lower forces and stretched less compared to formulation where 7mL of glycerol or triethanolamine (formulation B and D in Figure 3.9 and Table 3.5) was incorporated. Fibres where glycerol was added as a plasticiser (formulation D and E in Figure 3.9 and Table 3.5) were stronger and more flexible than the fibres where triethanolamine (formulations B and C in Figure 3.9 and Table 3.5) was added. From this study, glycerol was selected as the plasticiser for incorporation in alginate based monolithic fibres.

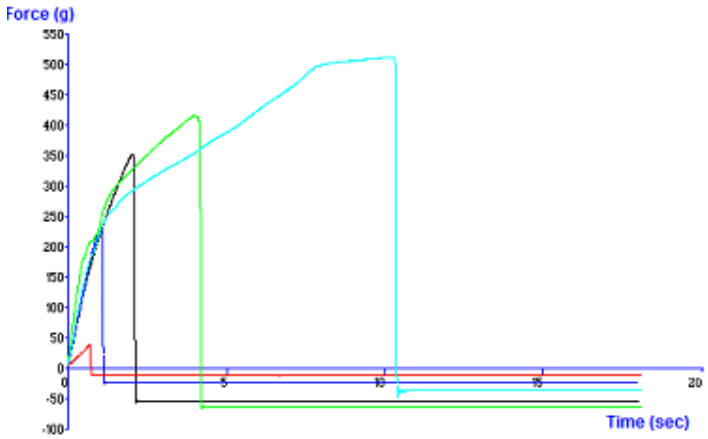


Figure 3.9: Typical force (g) vs. time (sec) for fibre formulations ─ A, ─ B, ─ C, ─ D and ─ E

Table 3.5: Comparison of breaking force and extension distance of fibres incorporating triethanolamine or glycerol as a plasticiser (N=5)

Formulation	*Breaking force (g)*	*Extension distance (mm)*
A	294.3±53.45	3.51±0.35
B	287.97±20.64	5.74±1.41
C	238.03±127.58	4.74±2.27
D	540.47±54.01	19.24±5.82
E	356.43±28.27	8.50±3.06

3.4.3. Drug entrapment efficiency of antimicrobial-loaded fibres

The calibration curves, Figure 3.10a and b, for ciprofloxacin and metronidazole respectively were conducted in crosslinking solution and used to determine the quantity of drug that was entrapped within the fibre.

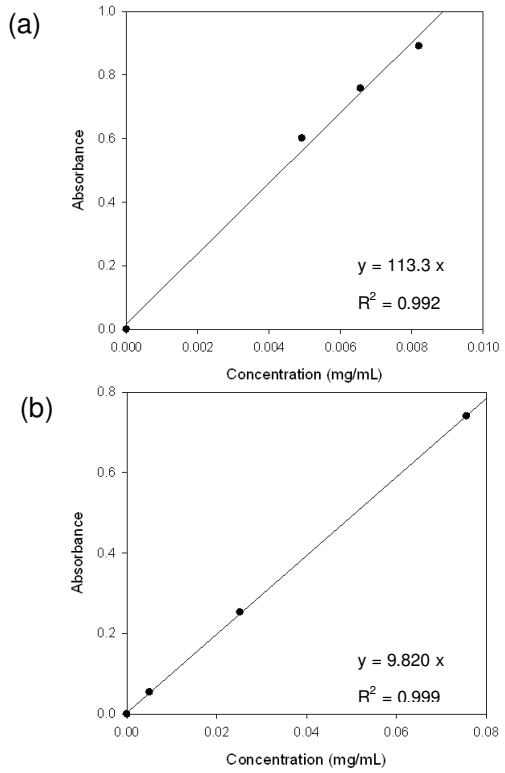


Figure 3.10: Calibration in crosslinking solution for (a) ciprofloxacin and (b) metronidazole

The amount of ciprofloxacin and metronidazole entrapped within the fibres was 80.06%±0.06 and 19.52%±0.27 respectively. Metronidazole readily diffuses from the fibre into the crosslinking solution resulting in only a small quantity of drug being entrapped within the fibre, compared to ciprofloxacin. The SEM images reveal that a similar shape and structure of the fibres (Figure 3.11) is maintained following the addition of glycerol and either ciprofloxacin or metronidazole as model antimicrobial agents compared to drug-free crosslinked alginate fibres (Figure 3.8). The diameter increases slightly to 510.1µm for ciprofloxacin-loaded (Figure 3.11a) and 594.8µm metronidazole-loaded (Figure 3.11b) fibres.

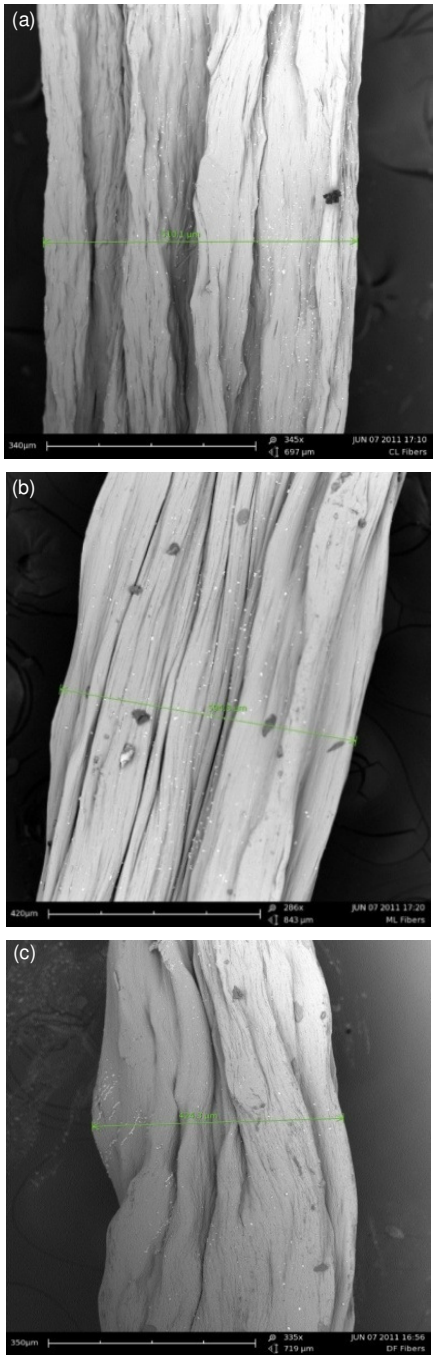


Figure 3.11: SEM images of (a) ciprofloxacin-loaded (diameter 510.1µm) and (b) metronidazole-loaded (diameter 594.8µm) and (c) drug-free (diameter 424.3µm) fibres

3.4.4. Antimicrobial assays for ciprofloxacin- and metronidazole-loaded fibres

Ciprofloxacin-loaded fibres demonstrated activity against both pathogens indicated by a clear zone of inhibition (Figure 3.12a and b). Similarly the ciprofloxacin-loaded disc was active against both pathogens (Figure 3.12e and f). Metronidazole-loaded fibres showed no activity against either *E. coli* or *E. faecalis* (Figure 3.12c and d). Likewise the metronidazole-loaded disc showed no activity against either pathogen (Figure 3.12e and f). No zone of inhibition surrounded the drug-free fibres (Figure 3.12a-d), which served as a further control, confirming that the polymeric fibre has no inherent antimicrobial activity against these pathogens.

The resistance of *E.coli* and *E. faecalis* to metronidazole exhibited in the drug-loaded fibres is similar to the literature results found. A study conducted by Onderdonk *et al.* (1979) confirmed that metronidazole did not demonstrate activity against *E. coli* in both animal models used and *in vitro* cultures tested. Investigation of *E. faecalis* activity against metronidazole revealed that *E. faecalis* demonstrates not only resistance to metronidazole, but inactivates the activity of the antibiotic against *Bacteroides fragilis* (Nagy and Földes, 1991). Ciprofloxacin is a bactericidal antibiotic with a wide spectrum of activity against Gram-positive and Gram-negative pathogens and demonstrated activity against both pathogens when loaded within the polymeric fibre.

Testing the fibres against causative pathogens was not possible as these periodontal pathogens were not available. Therefore, the model antimicrobial to be incorporated in the fibres needed to demonstrate activity against *E. coli* and *E. faecalis*. This study confirms that metronidazole is not active against these two pathogens and therefore should not be used as the model antimicrobial drug for further investigation.

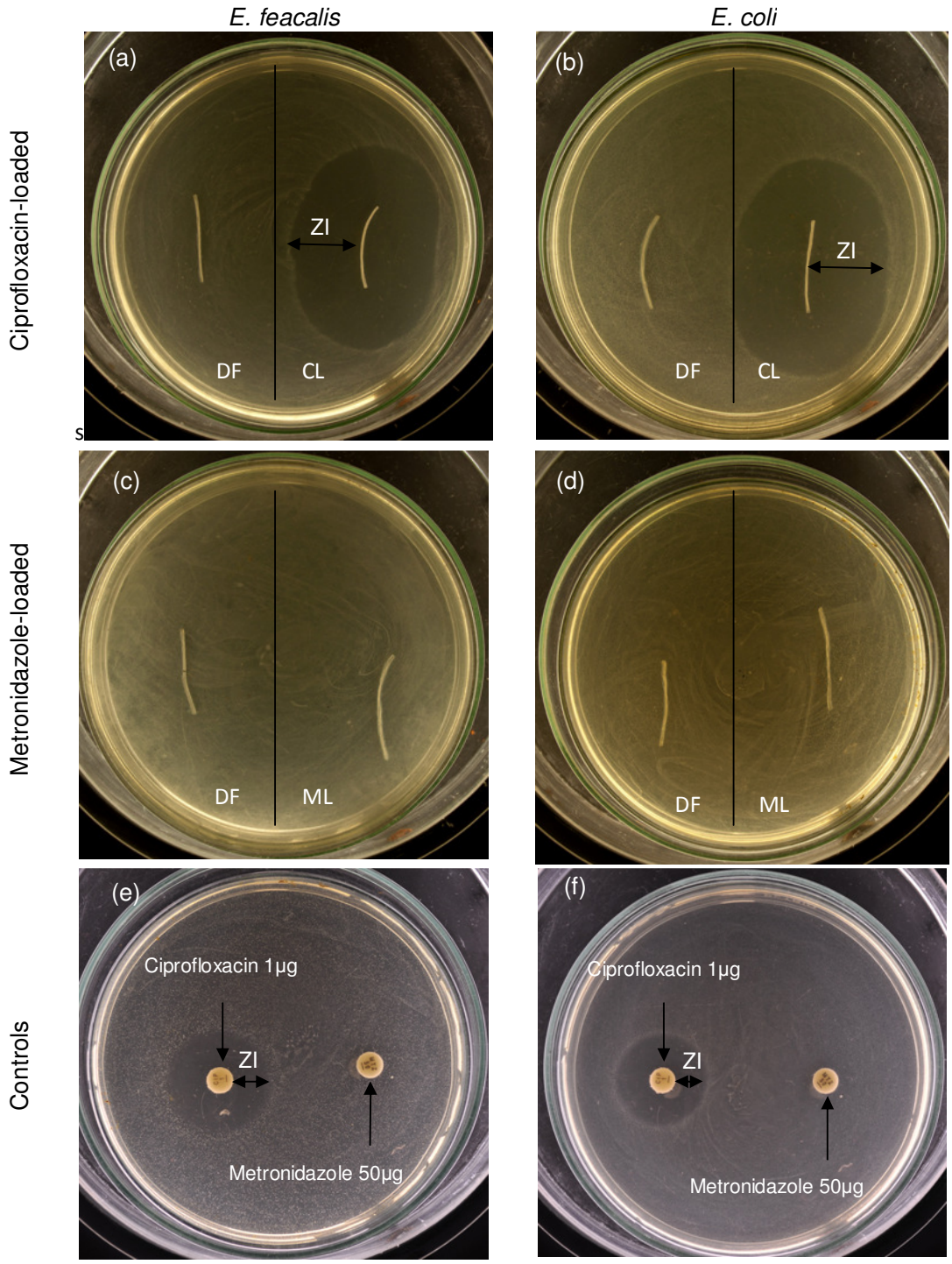


Figure 3.12: Antimicrobial activity represented by the zone inhibition (ZI) of ciprofloxacin-loaded (CL) and metronidazole-loaded (ML) fibres compared to controls; drug-free fibres (DF) and antimicrobial disks, against *E. coli* and *E. faecalis*

3.4.5. Dissolution studies

Standard USP (1995) apparatus and tests for dissolution would not adequately mimic the periodontal pocket environment where the fibres would be placed. To date there is no set dissolution methodology which mimics the periodontal pocket. This is illustrated in Table 3.6 which describe ten *in vitro* studies conducted on various local drug delivery devices for placement in the periodontal pocket. In each study the volume and pH of the dissolution medium where the device was placed as well as in the degree of agitation the device was exposed to while in the dissolution apparatus was different.

It has been reported that the pH of a single periodontal pocket ranges from 2 to 9 (Galgut, 2001). This creates distinctly different chemical environments and given that alginate displays pH responsive behaviour (Takka *et al.*, 1994; Al-musa *et al.*, 1999; Pillay and Fassihi, 1999), dissolution tests were conducted in PBS pH 4 and 6.8. In an attempt to create a pocket-like environment fibres were placed in the dialysing tube as described in a study conducted by Mundargi *et al.* (2007). The dialysing tube was tied at either end with nylon thread to prevent the escape of the fibre and then submerged in PBS. The dialysing tube used freely allows the passage of compounds with a molecular weight less than 12 000g/mol. Ciprofloxacin with a molecular weight of 331.35g/mol would easily pass through the membrane.

Table 3.6: Comparison of *in vitro* dissolution test apparatus for drug delivery systems intended for placement in the periodontal pocket

Archetype	*Dissolution medium*	*pH*	*Degree of agitation*	*Reference*
Film	10mL PBS	7.4	Rotating (15 rpm)	Park *et al.*, 1997
Wafer	1mL distilled water or human serum	*NS	Rotating (250 rpm)	Bromberg *et al.*, 2000
Chip	1mL distilled water (using Eppendorf tubes)	6-6.5	Rotating	Yue *et al.*, 2004
Hydrogels	35mL distilled water	*NS	Stirred with magnetic stirrer	Bako *et al.*, 2008
Microspheres	50mL PBS (placed in dialysis tube)	7.4	Rotating (50 rpm)	Mundargi *et al.*, 2007
Film	100mL of PBS / citrate buffer	3.5 and 7.4	Rotating	Wang *et al.*, 2007
Ointment	20mL PBS (thermostated flow-through erosion cell)	7.4	Flow rate 10mLhr^{-1}	Roskos *et al.*, 1995
Film	1mL PBS	6.6	Static	Ahmed *et al.*, 2009
Disk	10mL PBS	7.4	Rotating (65 rpm)	Johnson and Uhrich, 2009
Nanofibres	10mL PBS	7.4	*NS	Zamani *et al.*, 2010

*NS – not stated

Dissolution samples were tested in PBS at both pH 4 and pH 6.8; therefore calibration curves were constructed at both pH's. Calibration curves (Figure 3.13) were used to determine the amount of ciprofloxacin which had been released at each time point.

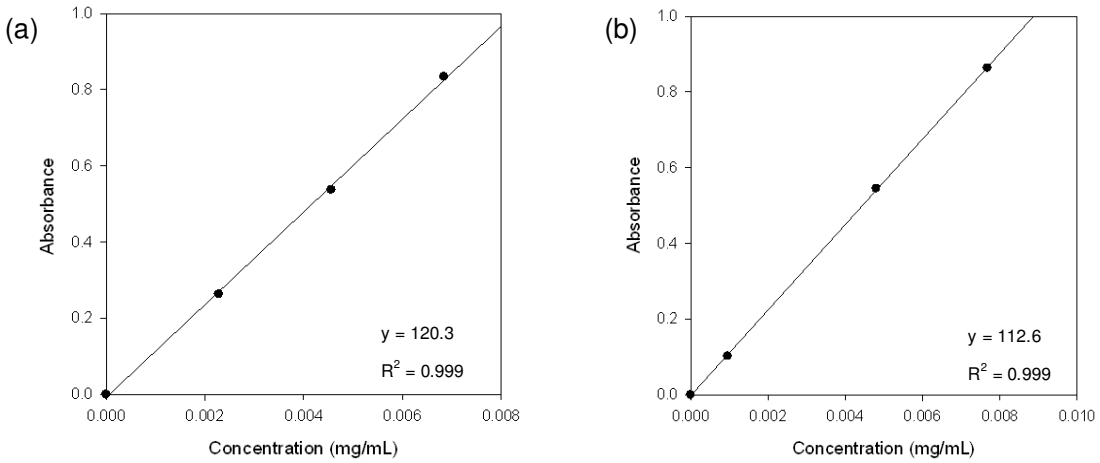


Figure 3.13: Calibration curve ciprofloxacin (λ_{278nm}) in PBS (a) pH 4 and (b) pH 6.8

The dissolution results confirmed the controlled release of ciprofloxacin from crosslinked alginate fibres with or without plasticiser at both pH 4 and 6.8 (Figure 3.14). A burst release of ciprofloxacin is seen in PBS pH 4 releasing 39.43% and 46.74% from alginate crosslinked fibres and plasticised alginate crosslinked fibres respectively in the first 24 hours. Alginate crosslinked fibres in PBS 6.8 release ciprofloxacin at a more controlled rate, releasing 16.31% in the first day, however, over 10 days more than half the drug remains entrapped within the fibre. Ciprofloxacin released from plasticised alginate crosslinked fibres in PBS pH 6.8 resembles a zero-order release pattern, with an average of 71.28% of drug released over the test period.

The results of the dissolution confirmed that glycerol had a noteworthy effect on ciprofloxacin's release. In both PBS pH 4 and pH 6.8 ciprofloxacin released faster from the plasticised fibres when compared to the non-plasticised formulations. Studies conducted on formulations where a plasticiser was added confirmed that zero-order delivery may be achieved when incorporating a suitable plasticiser (Pongjanyakul and Puttipipatkhachorn, 2007) as well as the presence of a plasticiser affects the permeability of the matrix (Qussi and Suess, 2006).

Changes in the morphological structure of the fibres were assessed in SEM images of fibres following the 10-day dissolution test in PBS pH 4 and 6.8 (Figure 3.15). Examination of the fibres in PBS pH 4 showed that the shape of the fibre remains intact following dissolution

(Figure 3.15a-d). Therefore, drug must have diffused from the matrix into the surrounding fluid. Relaxation and swelling of the polymeric matrix is evident in Figure 3.15e-h representing samples which underwent dissolution at pH 6.8. Furthermore, the flakes or pieces attached to the surface of the fibres are indicative of surface erosion. This analysis confirms that drug release in PBS pH 6.8 occurs as a result of swelling and erosion leading to disruption of the polymeric matrix. Tønnesssen and Karlsen (2002) describe the release of drug from calcium chloride crosslinked alginate as a "swelling–dissolution–erosion process" thereby modulating drug release. However, a crosslinked alginate forms an insoluble matrix in an acidic medium releasing the drug via diffusion mechanisms.

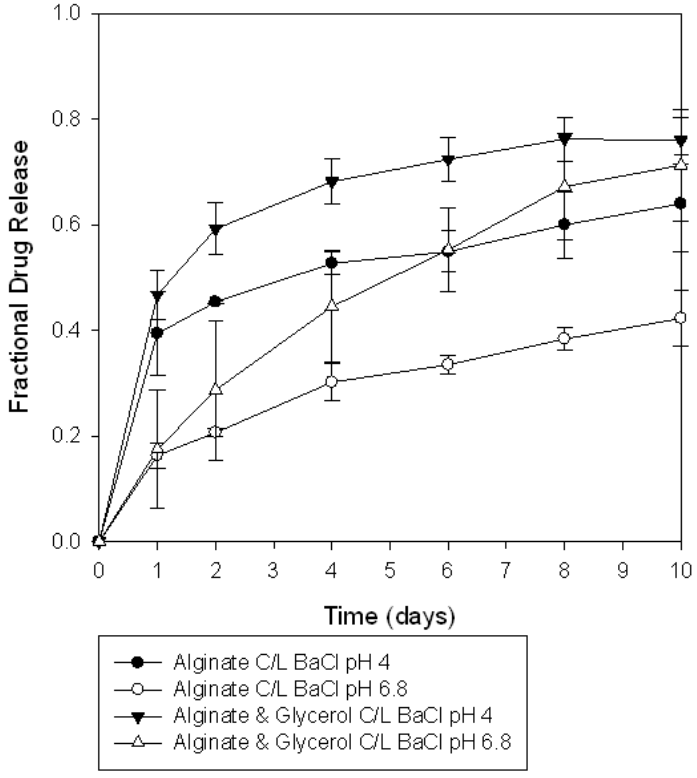


Figure 3.14: Fractional release of ciprofloxacin from crosslinked alginate fibres with and without plasticiser at pH 4 and pH 6.8

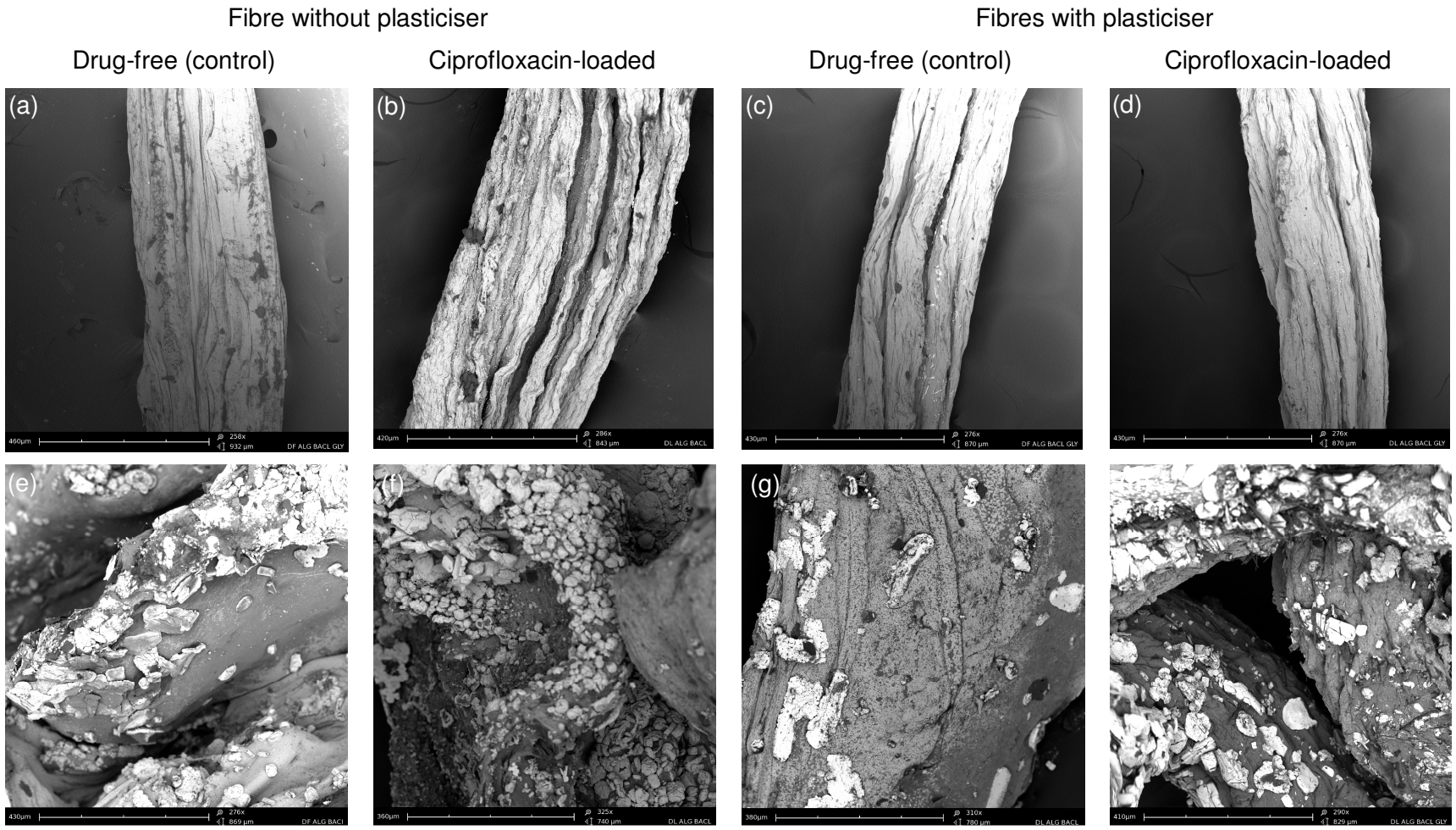


Figure 3.15: SEM images of fibres dried following a 10-day dissolution in PBS pH 4 and pH 6.8

3.4.6. Tensile analysis

The crosslinked plasticised alginate fibres were designed for placement within the periodontal pocket either around the tooth or overlapping on itself filling the pocket. As mentioned in Chapter 2 fibre placement is difficult, therefore necessitating tensile analysis to determine the fibres robustness. A suitable method to determine the tensile strength and flexibility of the fibres was determined through creep-, tensile- and dynamic-load analysis. The system used allows for precision testing of small-scale materials with an adjustable X-Y-Z axis and is free from external disturbances (Solomon, 2009).

Stereomicrographs (Figure 3.16) illustrated that although the fibres appear to be cylindrical in shape they have an irregular cross-sectional area. The average cross-sectional area was calculated at 0.338±0.028mm^2.

The preliminary fibres were assessed using creep-, tensile- and dynamic-loads. Typical strain and displacement profiles for fibres in Figure 3.17 allow for comparison of these three tests. The different load settings were compared to develop a suitable method which would quantify the strength and flexibility fibres and allow for easy comparison of different formulations when an experimental design would be employed.

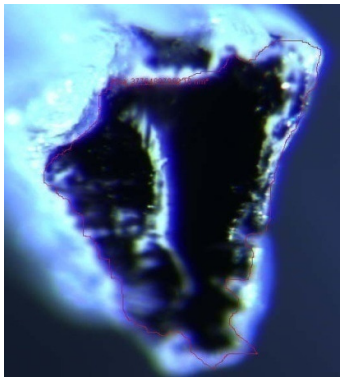
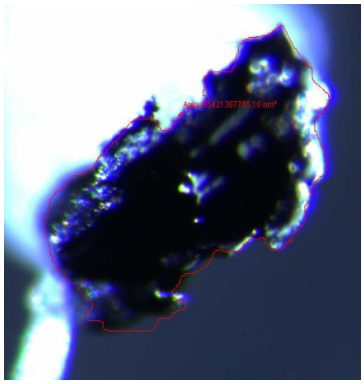
(a)  (b) 

Figure 3.16: Stereomicrographs depicting the cross-sectional area of crosslinked alginate plasticised fibres (a) 0.377mm^2 and (b) 0.354mm^2

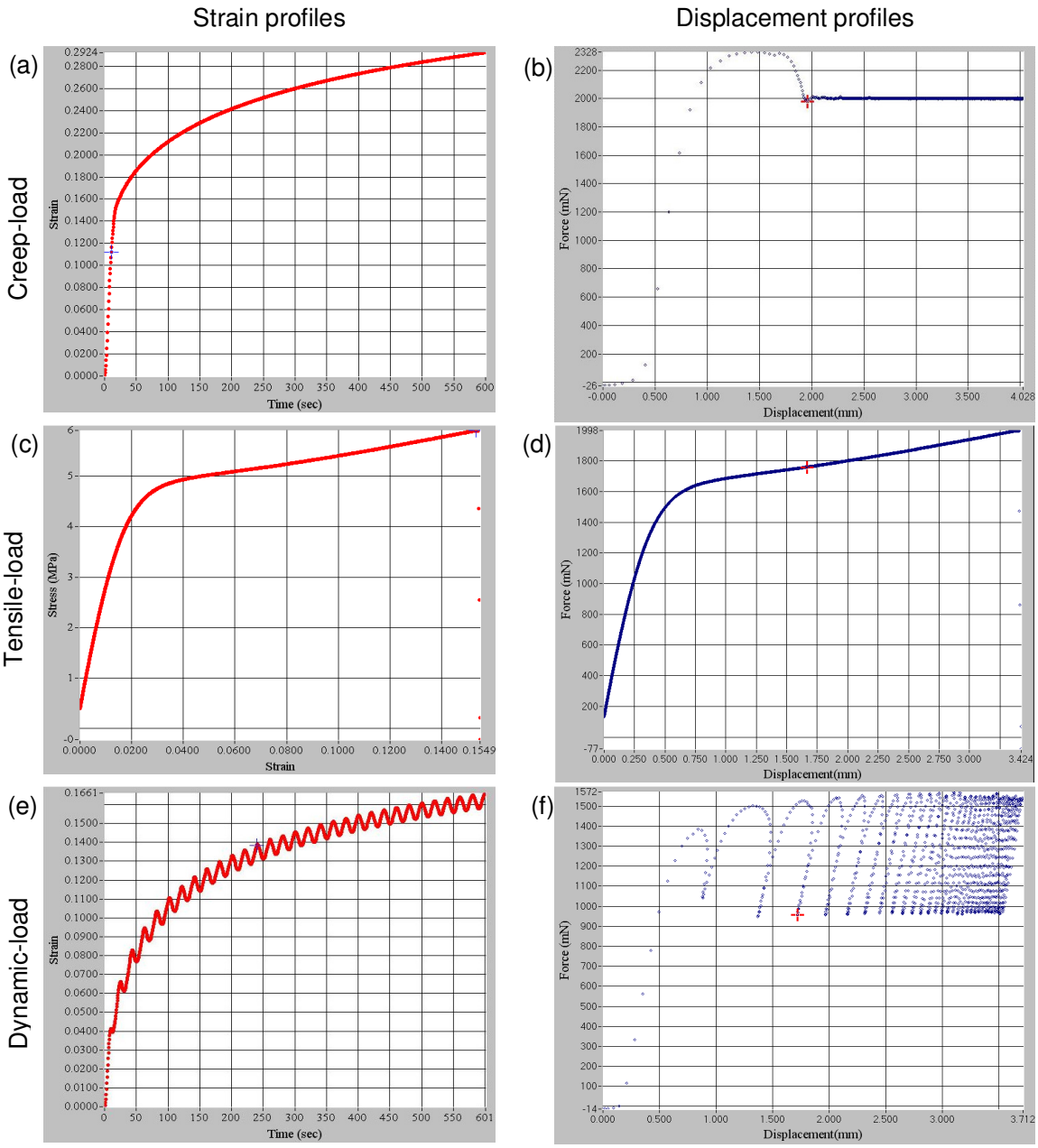


Figure 3.17: Typical strain and force vs. displacement curves for creep-, dynamic- and tensile-load analysis of fibre

3.4.6.1. Creep-load analysis

In the creep-load analysis the force was held at a constant value measuring the length the fibre would stretch to as shown in the typical strain and displacement profiles in Figure 3.17a and b. The results for fibres were assessed at 2000 and 2100mN are contained in Table 3.7. When fibres were held at a constant-load of 2000mN, two of the 5 fibres samples did not break over the 10 minute testing period. The maximum force which the test reached varied from 2268-2658mN with samples stretching between 4.03-10.76mm in length. In comparison, only one sample remained intact after 10 minutes when the load limit was increased to 2100mN, while the maximum force and displacement were similar at 2379-2656mN and 7.00-10.80mm respectively. The large variation within the five samples tested at both 2000mN and 2100mN, limits the usefulness of creep-load analysis for the comparison of data.

Table 3.7: Creep-load analysis of fibres at constant load limit of 2000 and 2100mN with the applied stress at 5.92MPa and 6.21MPa respectively (N=5)

Load limit (mN)	*Time till fracture (sec)*	*Strain (MPa)*	*Maximum Displacement (mm)*	*Maximum force (mN)*
2000	>600	0.29	6.01	2405
	38.5	0.42	9.00	2495
	159.0	0.31	6.36	2268
	37	0.62	10.76	2658
	>600	0.19	4.03	2328
2100	34	0.60	10.08	2379
	32	0.48	7.00	2522
	>600	0.37	7.85	2460
	41	0.85	7.91	2584
	33	0.55	9.83	2656

3.4.6.2. Dynamic-load analysis

Dynamic-load testing varied the load the fibre was exposed to and resulted in fluctuating strain and displacement profiles shown in Figure 3.17e and f. The results for dynamic-load analysis, included in Table 3.8, represented a fibre exposed to fluctuating loads between 1000 and 1500mN at 0.01Hz. Two of the samples did not fracture over the 5 minute test period. The number of cycles to fracture varied significantly from 0.34-3.00. The maximum strain was fairly constant (0.12-0.16MPa) for four of the samples test. Dynamic-load analysis revealed little information regarding the strength and flexibility of the fibres. Results between samples varied substantially. Furthermore, difficulties in setting of parameters led to wastage of samples.

Table 3.8: Dynamic-load analysis of fibres with oscillating load between 1000mN and 1500mN and the mean stress of 7.4MPa (N=5)

	Number of cycles till fracture	*Time to fracture (sec)*	*Maximum strain*
1	3.00	>300	0.16
2	0.46	46	0.68
3	0.34	30.5	0.15
4	1.28	123	0.12
5	3.00	>300	0.15

3.4.6.3. Tensile-load analysis

Tensile-load analysis of the fibres requires prior knowledge of standard engineering stress and strain terms. Figure 3.18 visually describes some of the terms used in analysing the tensile properties of the fibre. The fibre begins to deform as a force is applied, first undergoing elastic or reversible deformation, until a yield value is reached. Following which plastic or irreversible deformation occurs at higher stresses applied, finally leading to fracturing of the sample. The schematic (Figure 3.18) is similar in shape to the typical stress versus strain profile in Figure 3.17c.

Analysis of the tensile-load test calculates the Young's modulus, ultimate strength and strain, yield stress and toughness of the fibres (Table 3.9). Determination of the Young's modulus reveals information regarding the stiffness of the fibre and is defined as the ratio of stress over strain (Equation 3.5). The yield stress represents the value at which the fibre would plastically deform. Deformation beyond this point would lead to permanent non-reversible deformation. Ultimate strength represents the highest stress point measured. Flexibility of the fibres were analysed through determination of the ultimate strain. The fibres undergoes plastic deformation and the strain at which the fibre fractures is the ultimate strain value recorded. Toughness characterises the resistance of the fibre to fracture when stressed is applied and is calculated using the area under the curve.

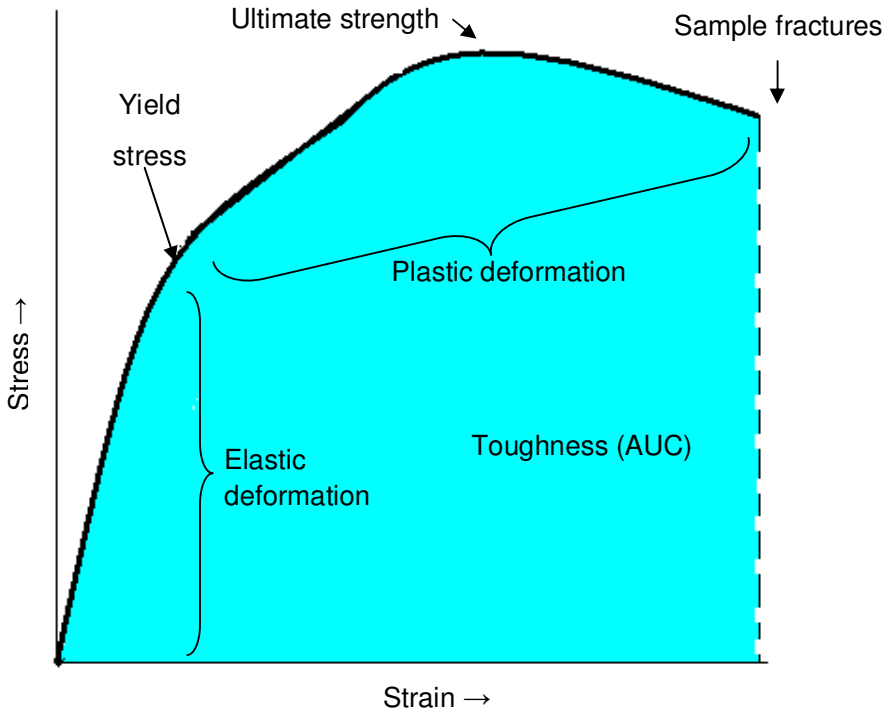


Figure 3.18: Schematic sketch of stress vs. strain profile (Adapted from Cooke *et al.*, 1996)

Table 3.9: Tensile-load analysis (N=5) of fibres at a constant velocity rate of 20µmsec^{-1}

	Young's modulus (MPa)	*Yield stress (MPa)*	*Ultimate strain*	*Ultimate strength (MPa)*	*Toughness (Jcm^{-3})*
1	218.85	3.99	5.91	0.15	0.76
2	258.50	4.75	7.07	0.12	0.65
3	280.63	5.28	6.80	0.08	0.45
4	251.87	3.38	4.44	0.053	0.14
5	270.70	5.51	7.79	0.087	0.52
Ave:	256.11± 23.59	4.58±0.89	6.40±1.29	0.098±0.04	0.50±0.24

The tensile-load analysis software uses engineering stress and strain equations specified in Equations 3.3 and 3.4 respectively. Equation 3.3 represents the determination of stress (N) where the force [F] measured in Newton's represents the instantaneous load applied and [A_0] measures the cross-sectional area prior to deformation. The strain, Equation 3.4 compares the change in deformation of the fibre to the original fibre, [l_i] and [l_0] representing the final and original length of the fibres, respectively. The Young's modulus is ratio of tensile stress (σ) to tensile strain (ε) as represented in Equation 3.5.

$$\sigma = \frac{F}{A_0} \qquad \textit{Equation 3.3}$$

$$\varepsilon = \frac{l_i - l_o}{l_o} \qquad \textit{Equation 3.4}$$

$$E = \frac{\sigma}{\varepsilon} = \frac{F\ Lo}{Ao\Delta L} \qquad \textit{Equation 3.5}$$

Determination of Young's modulus, yield stress, ultimate strength, ultimate strain and toughness allows for easy comparison of the extensibility and strength of the fibres. Therefore, tensile-load tests were used for further tensile analysis of the experimental design and optimised formulations.

3.4.7. Effect of formulation components on the formation of fibres

Fibres were prepared with varying concentrations of alginate, barium chloride and glycerol to determine the upper and lower limits which the Design of Experiments would be based upon. The polymer, crosslinking salt and plasticiser concentrations were identified as principle parameters in the forming of fibres. The upper and lower limits for each formulation component were determined (Table 3.10). Adequate fibres formed when the alginate and barium chloride was at a concentration between 1-4%$^{w}/_{v}$ and 0.5-10%$^{w}/_{v}$ respectively and incorporating 5-25mL of glycerol.

Table 3.10: Effect of formulation components alginate, barium chloride and glycerol on observed fibre formation

Alginate ($\%^{w}/_{v}$)	*Barium chloride* ($\%^{w}/_{v}$)	*Glycerol* *(ml/25mL polymer solution)*	*Remark*
0.25-0.5	2.00	7	Fibres formed web
1.00-3.00			Adequate fibres formed
4.00			Very viscous polymer solution
>4.00			Solution too viscous
2.00	0.125-0.25	7	Fibres formed clumps
	0.50-10.00		Adequate fibres formed
	>10.00		No difference in fibre formation
2.00	2.00	<5	Too brittle
		5-25	Adequate fibres formed
		>25	Flat shape

3.5. Concluding Remarks

This chapter aimed to design a PFD which could potentially be used in the treatment of PD. The monolithic fibre formulated was assessed according to drug entrapment, antimicrobial activity, drug release and tensile strength. The tests aided in choosing a suitable plasticiser and antimicrobial for incorporation within the fibre as well as setting the upper and lower limits of the formulation components for the experimental design.

Brittle fibres were formed when alginate was crosslinked in a barium chloride solution. Investigations revealed that glycerol was a suitable plasticiser, which would increase the strength and flexibility of the crosslinked fibres. Tensile analysis of preliminary fibres investigated the creep-, tensile- and dynamic-loads. Creep- and dynamic-load tests provided limited quantitative information regarding the tensile properties of the fibres. The results were difficult mean in order to characterise the formulation. The variable parameter settings meant that the test needed adjustment for each sample tested. In comparison, tensile-load analysis provided valuable information which was used in the quantitative comparison of the experimental design formulations.

Qualitative antimicrobial analysis and determination of the drug entrapment indicated that ciprofloxacin was active against both *E. coli* and *E. faecalis* as well as having a notably higher drug entrapment within the PFD. This provided the motivation for incorporation of ciprofloxacin as the model antimicrobial.

Controlled drug release was evident in crosslinked alginate formulations, with or without plasticiser, tested at pH 4 and 6.8. Drug release of ciprofloxacin from fibres was pH responsive, which correlated with the literature surveyed. Upon examination of SEM images with dissolution data, drug release at pH 4 occurs as a result of drug diffusing through the polymeric matrix, however, at pH 6.8, disruption of the fibre structure leads to drug release as a consequence of the swelling and erosion of the matrix. Ciprofloxacin is released faster from fibres following incorporation of glycerol. Promising dissolution results were obtained from PFD, with zero-order release at pH 6.8 from the plasticised alginate fibres.

Polymer, crosslinking salt and plasticiser concentrations were identified as the noteworthy factors affecting fibre formulation. The upper and lower limits were determined as 2-4%$^{w}/_{v}$, 0.5-10%$^{w}/_{v}$ and 5-25mL for alginate, barium and glycerol respectively. These formed the bases of the experimental design which was elaborated upon in Chapter 4.

CHAPTER 4

FABRICATION AND OPTIMISATION OF A POLYMERIC FIBRE DEVICE

4.1. Introduction

Pharmaceutical technology has progressed from traditionally changing one variable at a time to a systemic approach, utilising a Design of Experiments (DoE) demonstrating the following advantages (Singh *et al*., 2004; Singh *et al*., 2005):

- Reduced number of experiments.
- Problem tracing and rectification.
- Identifying drug / polymer interactions.
- Simulate product performance using model equations.
- Evaluate processes leading to improved product development and scale-up production.

In a multifactor approach the variables are changed together rather than one at a time. This provides pertinent information regarding the magnitude and nature of interactions between variables as well as obtaining a measure of experimental error. Box and Behnken (1960) devised a statistical based experimental design where multiple variables may be changed simultaneously. A stepwise approach delineated in Figure 4.1 can be employed in the optimisation of pharmaceutical formulations. Numerous extended release products intended for intraoral application have used a DoE approach to optimise the formulation employing a 3^2 full factorial design (Bukka *et al*., 2010; Narendra *et al*., 2005; Wong *et al*., 1998). A 3-Factor Box-Behnken Design, which generates 15 experimental design formulations with 3 centre points as sketched in Figure 4.2, was the design upon which the PFD for this study was built.

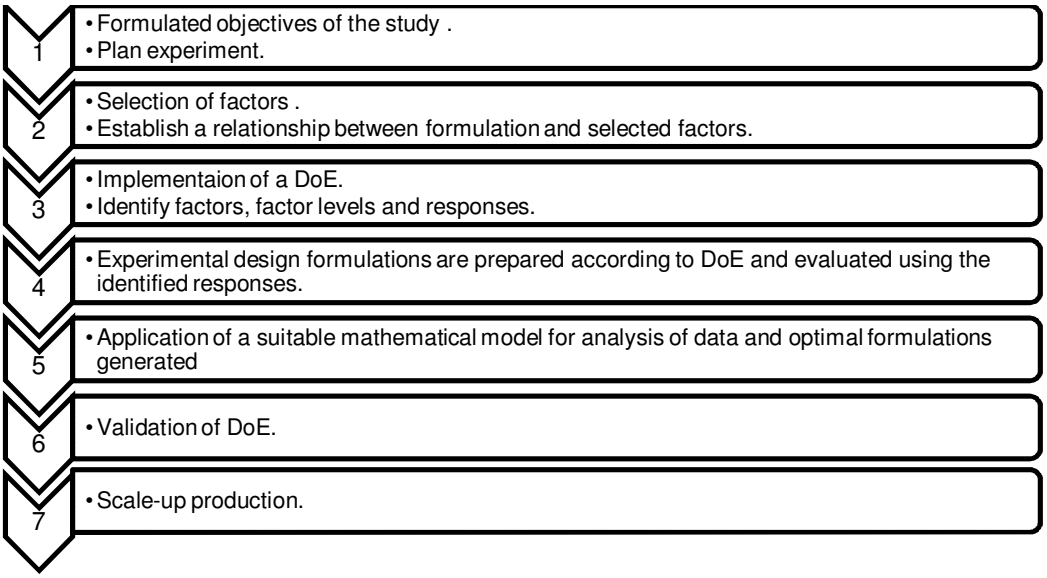


Figure 4.1: Schematic outline of the DoE process (Adapted from Singh *et al.*, 2004)

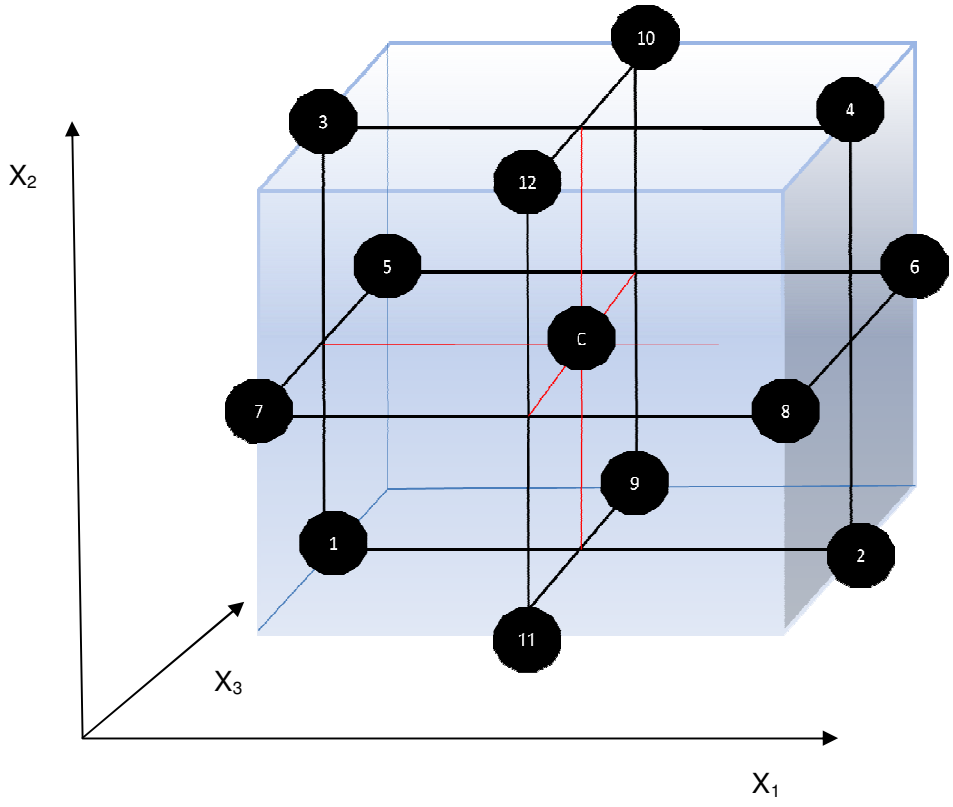


Figure 4.2: A combined cube with three interlocking 3^2 factorial design (Adapted from Ferreira *et al.*, 2007)

The schematic, Figure 4.1, suggests a process specific approach to the DoE in the formulation of pharmaceutical products (Singh *et al*., 2004). As outlined, steps 1-6 are necessary in the optimisation of the PFD. Thus far, the formulation process and variables have been identified. Preliminary studies described in Chapter 3 investigated possible variables such as formulation components, component concentrations and formulation processes. Polymer, crosslinking salt and plasticiser concentrations where isolated as variables possibly possessing a notable effect on the overall mechanism of operation of the fibres. It was anticipated that optimisation of the PFD would enhance drug release over 10 days and ensure that the mechanical properties of the fibre met the intended criteria for easy placement within the periodontal pocket. Hence, proposed responses to be measured, focused on the *in vitro* drug release profiles and mechanical properties of the co-blended polymeric fibre.

4.2. Materials

Materials used were obtained as outlined in Chapter 3 Section 3.2. Diclofenac sodium was obtained from Sigma-Aldrich, St Louis, Missourri.

4.3. Methods

4.3.1. Formulation preparation using a 3-Factor Box-Behnken Design

The experimental design fibre formulations were prepared by varying the polymer, crosslinking salt and plasticiser concentration. The upper and lower limits, as indicated in Table 4.1, formed the factors upon which the design was built.

Table 4.1: Upper and lower limits of the independent variables; polymer, crosslinking salt and plasticiser concentration

Independent Factors	*Lower level*	*Upper level*
Polymer concentration (X_1): Sodium Alginate ($\%^{w}/_{v}$)	2	4
Crosslinking salt concentration (X_2): Barium Chloride 2-hydrate ($\%^{w}/_{v}$)	0.5	10
Plasticiser concentration (X_3): Glycerol (mL/25mLpolymer solution)	5	25

A Box-Behnken design, generated by Minitab® V14 (Minitab®, USA), was employed to statistically optimise the fibre formulation. A 3-factor design provided 15 experimental formulations varying the sodium alginate (ALG), glycerol (GLY) and barium chloride 2-hydrate (BaCl) concentrations as shown in Table 4.2.

Table 4.2: The 3-factor Box-Behnken Design generated for optimisation of the fibres

Experimental design formulation	*ALG* ($\%^{w}/_{v}$)	*BaCl* ($\%^{w}/_{v}$)	*GLY* (*mL/25mL*)
1	2.00	10.00	15.00
2	4.00	5.25	25.00
3	2.00	5.25	25.00
4	3.00	5.25	15.00
5	3.00	10.00	5.00
6	4.00	10.00	15.00
7	2.00	5.25	5.00
8	3.00	5.25	15.00
9	3.00	0.50	5.00
10	4.00	0.50	15.00
11	3.00	0.50	25.00
12	3.00	5.25	15.00
13	3.00	10.00	25.00
14	2.00	0.50	15
15	4.00	5.25	5.00

4.3.2. Preparation of PFD

Polymeric based monolithic fibres were prepared using various combinations of alginate (2-4%$^{w}/_{v}$), barium chloride (0.5-10%$^{w}/_{v}$) and glycerol (5-25mL) according to the 15 experimental design formulations generated in the 3-factor Box-Behnken design. The fibres were prepared as in Chapter 3 Section 3.3.1 with each formulation prepared in triplicate; drug-free, diclofenac sodium-loaded (500mg) and ciprofloxacin-loaded (200mg) fibres. For drug-loaded formulations the model drug was dispersed in distilled water prior to the addition of alginate.

4.3.3. Determination of drug entrapment efficiency

Drug entrapment was determined through analysis of the drug remaining in the crosslinking solution once the fibres were removed, as described in Chapter 3 Section 3.3.4. The entrapment was calculated separately for diclofenac sodium and ciprofloxacin for each of the 15 experimental designs formulations. The amount of drug in the crosslinking solution was determined by UV at λ_{max}=278nm for ciprofloxacin or λ_{max}=276nm for diclofenac sodium.

4.3.4. *In vitro* drug release studies

The dissolution apparatus and methodology was the same as described in Chapter 3 Section 3.3.6. Drug release of ciprofloxacin- and diclofenac-loaded fibres were tested individually for each of the experimental design formulations.

A pharmacokinetic model-independent approach was used to compare the dissolution data in PBS pH 6.8. The mean dissolution time (MDT) over 10 days was calculated as in Equation 4.1.

$$MDT = \sum_{i=1}^{n} ti \frac{Mt}{M\infty} \qquad \textit{Equation 4.1}$$

Mt is the fraction of dose released in time $ti = (t_i - t_{i-1}) / 2$ and $M\infty$ corresponds to the loading dose.

4.3.5. Tensile strength analysis

Mechanical properties of drug-free fibres were tested for each of the experimental designs and were assessed according to their tensile strength. Samples were prepared and loaded as outlined in Chapter 3 Section 3.3.7. The experimental design formulations were analysed using the tensile-load setting described in Chapter 3 Section 3.3.7.3.

4.3.6. Optimisation of design

The experimental design generated polynomial equations which were solved for the optimised formulation taking into consideration the output response variables for MDT, ultimate strain and Young's modulus. The predetermined constraints facilitated the generation of an ideal formulation. The design was assessed according to the degree of correlation between experimental and predicted data.

4.4. Results and Discussion

4.4.1. Preparation of the experimental design formulations

The first group of 15 formulations were prepared without drug. The second and third groups were prepared with ciprofloxacin and diclofenac sodium respectively. Drug entrapment and release behaviour studies were performed on the on ciprofloxacin- and diclofenc-loaded fibres while tensile strength analysis was conducted on drug-free fibres.

4.4.2. Drug entrapment of experimental design formulations

Calibration curves were determined for diclofenac sodium and ciprofloxacin in the drug-free crosslinking solution for each of the 15 experimental design formulations. Typical examples of a calibration curves are represented in Figure 4.3 and 4.4 for the experimental designs of formulation 3 for ciprofloxacin (λ_{278nm}) and diclofenac sodium (λ_{276nm}) respectively. Similar calibration curves were constructed for the remaining 15 experimental design formulations, however these were not included for the sake of brevity.

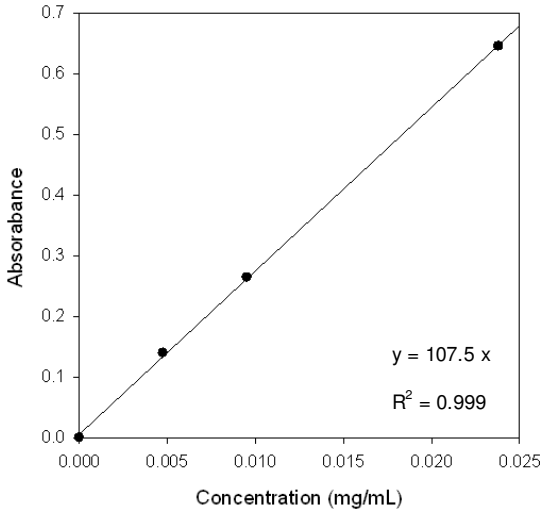


Figure 4.3: Calibration curve ciprofloxacin (λ_{278nm}) in curing solution prepared in formulating experimental design formulation 3

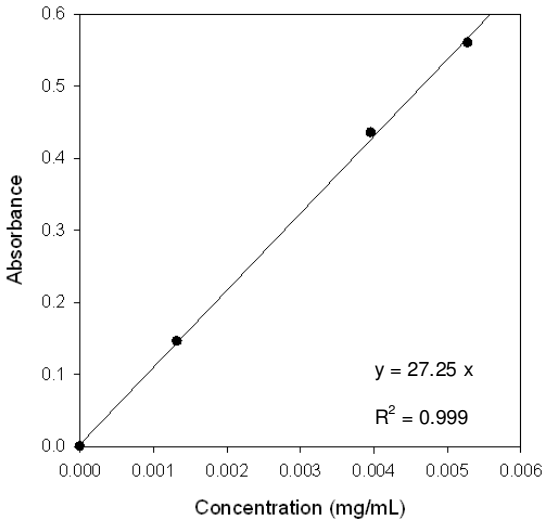


Figure 4.4: Calibration curve diclofenac sodium at (λ_{276nm}) in curing solution in formulating experimental design formulation 3

The average ciprofloxacin and diclofenac entrapment including standard deviations for each experimental design formulation are summarised in Figure 4.5. Drug entrapment for all formulations exceeded 60% both for ciprofloxacin and diclofenac sodium.

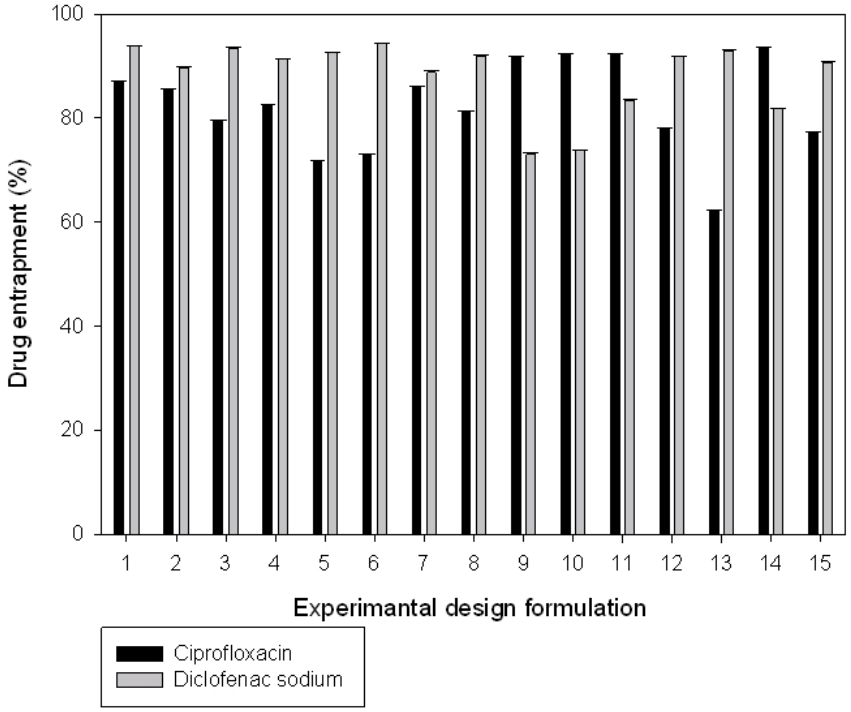


Figure 4.5: Vertical bar chart representing the percentage of ciprofloxacin and diclofenac sodium entrapped in the 15 experimental design formulations (N=3)

4.4.3. *In vitro* drug release

Drug release of ciprofloxacin and diclofenac sodium from the experimental design formulations were tested individually at pH 4 and 6.8. Dissolution results are shown in Figure 4.6-4.9. Each figure includes dissolution results for an experimental design formulation releasing ciprofloxacin and diclofenac in PBS at pH 4 and 6.8. One of the key parameters controlling drug absorption is solubility (Amidon *et al.*, 1995). The results obtained in the dissolution profiles reflect the solubility of ciprofloxacin and diclofenac in the respective dissolution media.

Diclofenac sodium is a weak acidic drug (pKa = 4.0) and is poorly soluble in acidic conditions. Therefore dissolution tests are normally conducted in media with a pH greater than 6.5 (Palomo *et al.*, 1999). The poor solubility is verified in the fractional drug release profiles at pH 4 in Figures 4.6-4.9. However, the rate and amount of diclofenac sodium released in PBS pH 6.8 was far greater. Pillay and Fassihi (1999) noted that diclofenac release from alginate beads crosslinked with calcium at pH 6.6 is dependent on drug solubility as well as matrix swelling and relaxation.

Reddy *et al.* (2011) calculated the intrinsic solubility constant for ciprofloxacin in 0.1N HCl as 6.55mg/cm^{2}min compared to 0.02mg/cm^{2}min in PBS pH 6.8. The increased solubility in acidic solutions was attributed to protonation of the piperazine ring. Ciprofloxacin is released faster and to a greater extent from fibres submerged in PBS pH 4 compared to PBS pH 6.8, demonstrated in Figures 4.6-4.9. However, the difference in release profiles is not as severe as diclofenac sodium released in PBS pH 4 and 6.8

Similar trends to the fractional release curves in Chapter 3 Section 3.3.5 are evident. Drug release from a crosslinked alginate matrix in an acidic medium is dependent on the drug diffusing from the matrix into the dissolution medium. Diclofenac sodium, poorly soluble in acidic solutions, is hardly released in PBS pH 4 compared to ciprofloxacin, which is highly soluble in acidic solutions. Both ciprofloxacin and diclofenac sodium are released in PBS pH 6.8, due to swelling and erosion of the crosslinked alginate fibre.

Drug release is an important factor that needs to be optimised in the DoE. As diclofenac was poorly released in PBS pH 4, it was decided that dissolution results in PBS pH 6.8 for both diclofenac sodium and ciprofloxacin would be used in the optimisation of the fibres. For accurate comparison of formulations the MDT over 10 days was calculated. A reduction in MDT indicates that the matrix retards drug release.

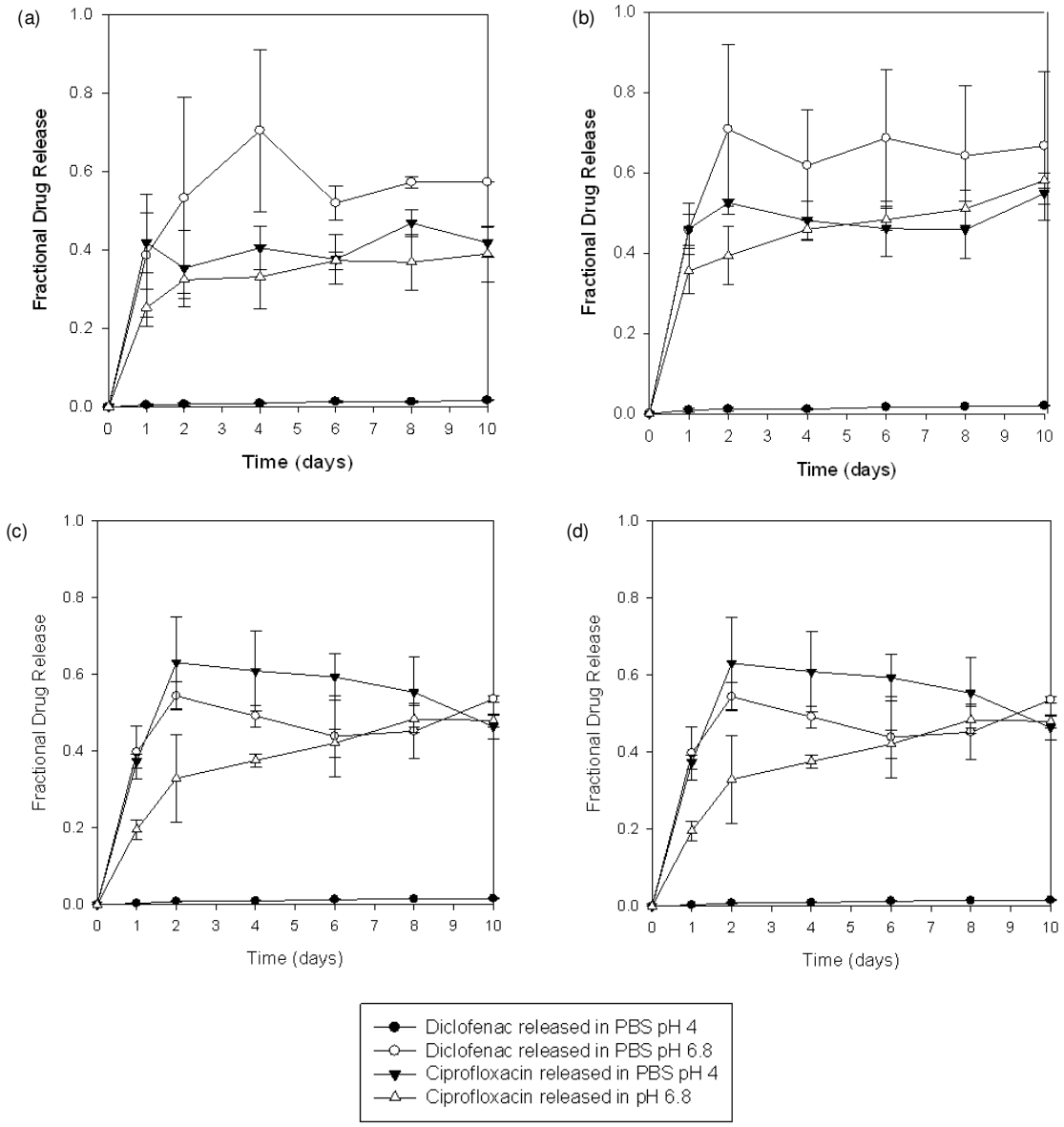


Figure 4.6: Drug release profiles for experimental design formulations 1 – 4 (a – d) (N=3)

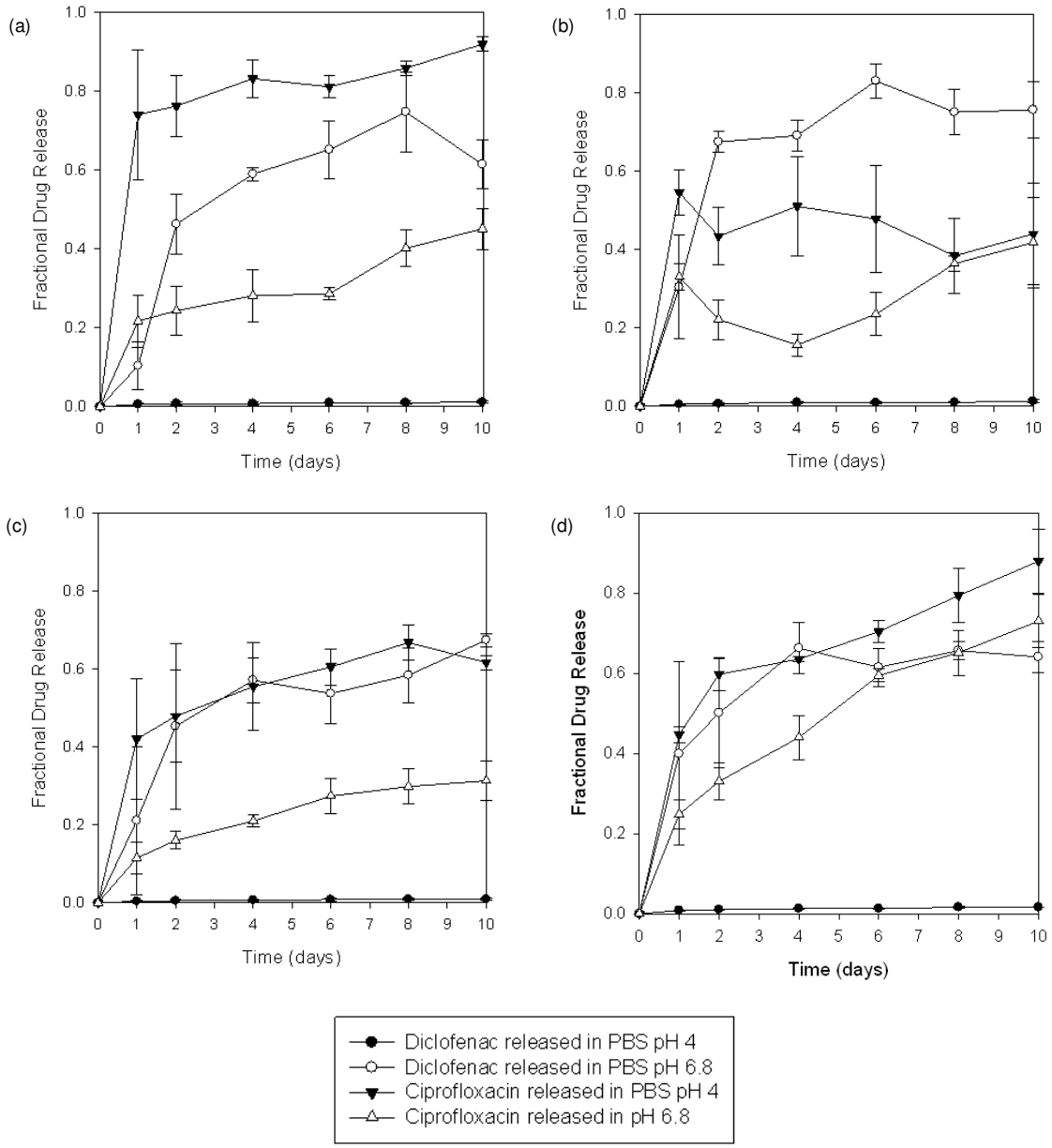


Figure 4.7: Drug release profiles for experimental design formulations 5 – 8 (a – d) (N=3)

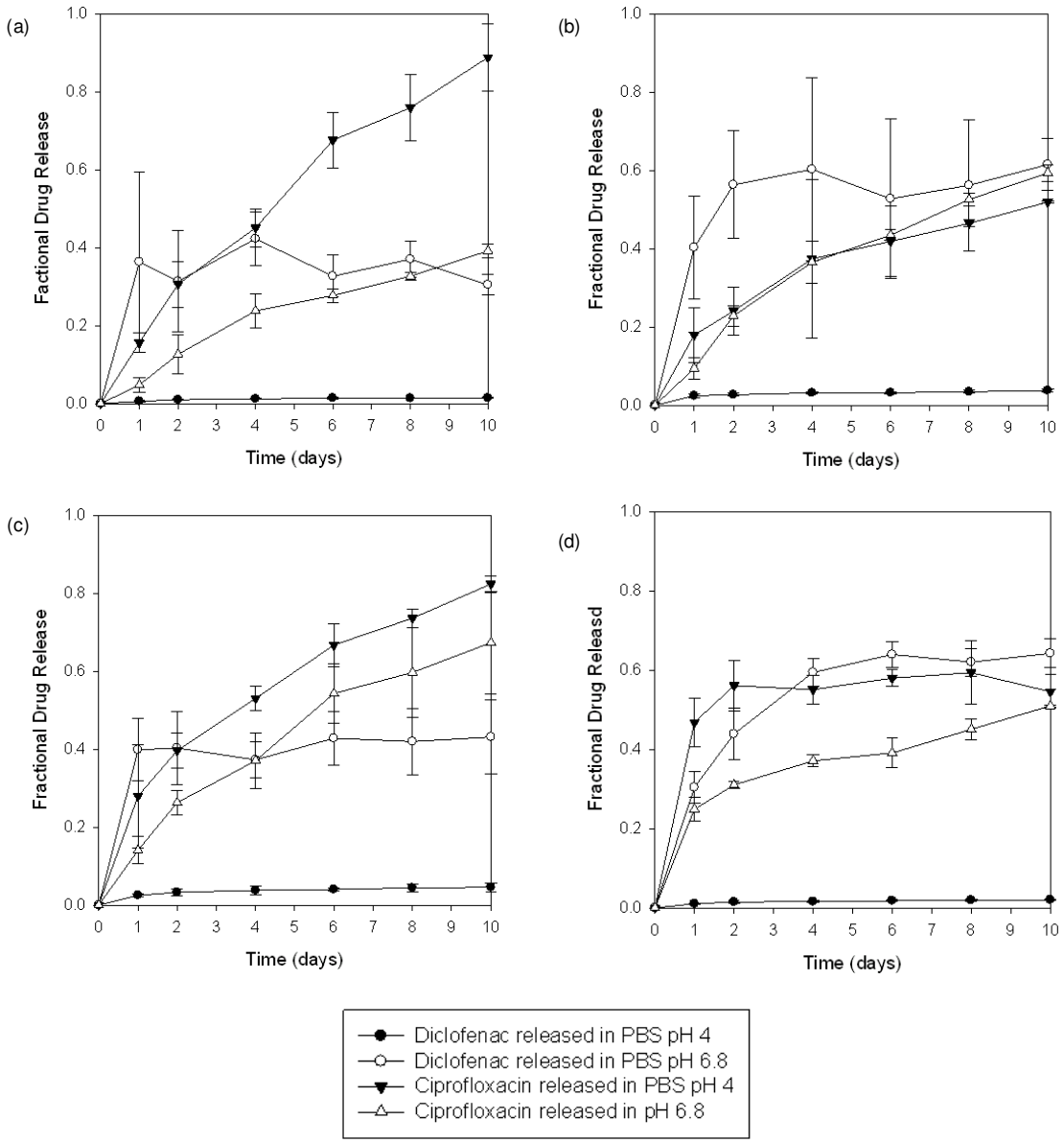


Figure 4.8: Drug release profiles for experimental design formulations 9 – 12 (a – d) (N=3)

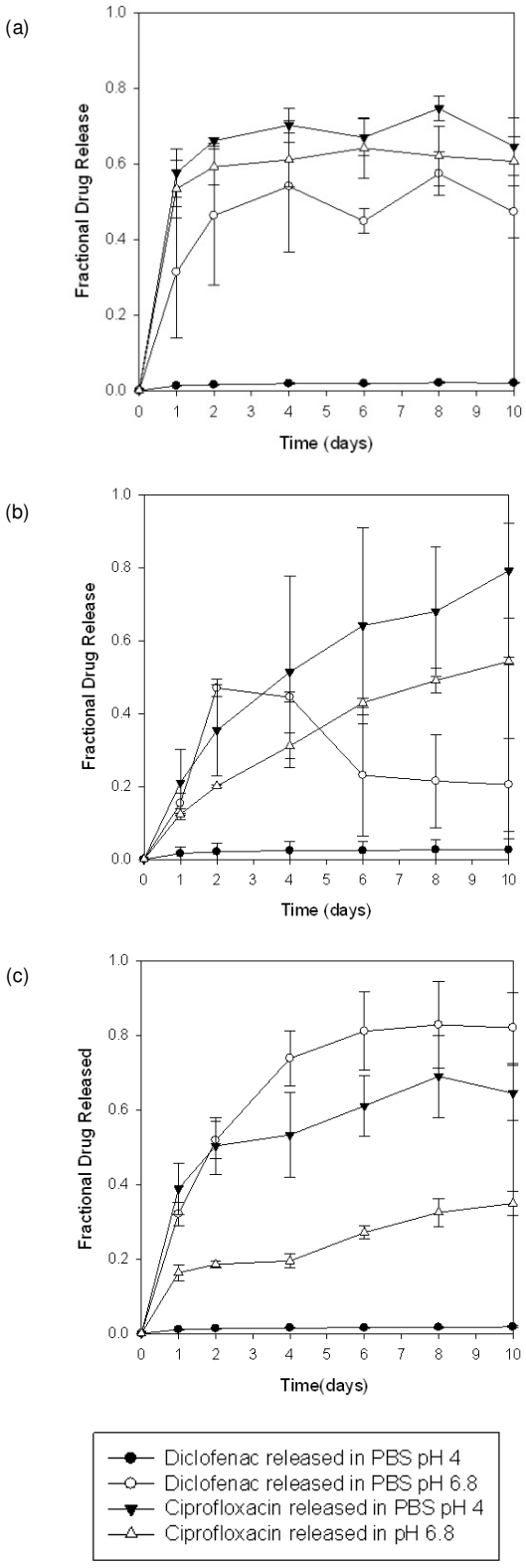


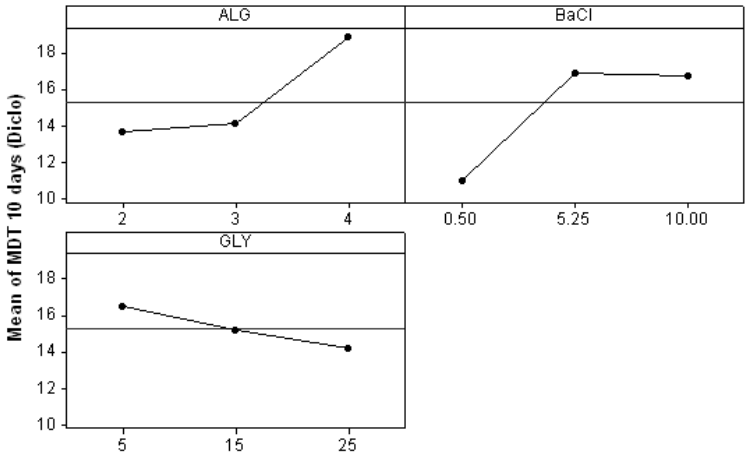
Figure 4.9: Drug release profiles for experimental design formulations 13 – 15 (a – c) (N=3)

Fibres were optimised using fractional drug release results for ciprofloxacin and diclofenac at pH 6.8. The MDT over 10 days for ciprofloxacin and diclofenac sodium released in PBS pH 6.8 was calculated as in Equation 4.3. Analysis of the main effects plots and interaction plots established a relationship between the formulation components and the drug release behaviour exhibited by the experimental designs (Figure 4.10 and 4.11). Calculation of the MDT indicated the effect of the polymer on the drug release rate from the formulation (Kuksal *et al.*, 2006).

High alginate ($4\%^{w}/_{v}$) and lower barium chloride ($0.5\%^{w}/_{v}$) concentrations had a noteworthy effect on respectively increasing and decreasing the $MDT_{10\ days}$ for diclofenac release (Figure 4.10a). In comparison the plasticiser had a notable influence on the $MDT_{10\ days}$ for ciprofloxacin release (Figure 4.11a), the MDT value increases with an increasing volume of glycerol added to the formulation.

Examination of the interaction plots, Figure 4.10b and 4.11b, determined the combined effects of two variables on MDT. Low polymer and crosslinking salt levels had the greatest effect on decreasing the $MDT_{10\ days}$ for diclofenac release (Figure 4.10b). The highest MDT_{10days} for diclofenac release was noted when a $4\%^{w}/_{v}$ alginate solution was crosslinked in a $10\ \%^{w}/_{v}$ barium chloride solution or when combined with minimum amount of glycerol (5mL) (Figure 4.10b). High levels of glycerol combined with a $4\%^{w}/_{v}$ alginate solution increased the $MDT_{10\ days}$ for ciprofloxacin substantially (Figure 4.11b).

(a)



(b)

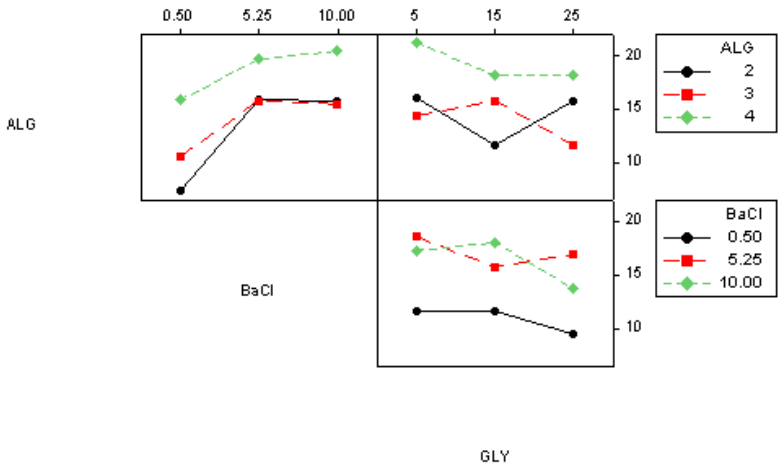
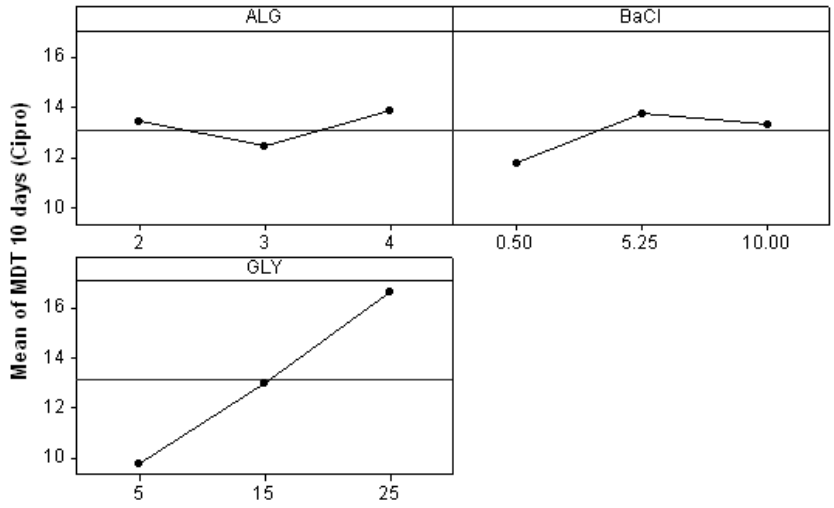


Figure 4.10: Factor plots, (a) main effects plot and (b) interaction plots, depicting the correlation between $MDT_{10\ days}$ for the release of diclofenac sodium and design variables

(a)



(b)

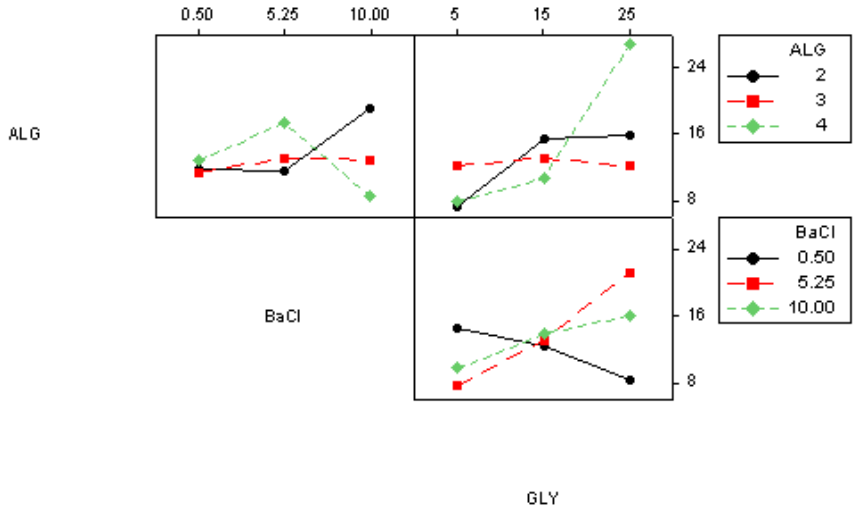


Figure 4.11: Factor plots, (a) main effects plot and (b) interaction plots, depicting the correlation between $MDT_{10\ days}$ for the release of ciprofloxacin and design variables

4.4.4. Tensile analysis

Tensile analysis is an important parameter in optimisation of fibres as it affects the manufacturing processes used for fibre production and placement of the fibre within the periodontal pocket. The periodontal pocket is a narrow sulcus which is formed as free gingiva moves away from the tooth surface, which is approximately 3mm deep in healthy gums and increases to 6mm in the presence of PD (Nield-Gehrig, 2007). This relatively small area poses challenges for placement of a device, therefore it is necessary to formulate a strong flexible fibre that is easily handled and placed within the pocket. Tensile analysis requires determination of the cross-sectional area of the fibres. The fibres appear cylindrical to the naked eye, however, examination of fibres using the stereomicroscope reveal an irregular shape. The cross-sectional area of the 15 experimental designs is contained in Table 4.3. Tensile-load analysis of the experimental design formulations allowed for comparison of the strength and flexibility of the formulations, which are compared in the vertical bar charts Figures 4.12-4.16. Young's modulus, yield stress and ultimate strength results (Figure 4.12-4.14) displayed a similar pattern and were a measure the tensile strength of the material. Concurrent analysis of these charts reveals that formulation 7 was the strongest formulation (Young's modulus 1814±96.33MPa; ultimate strength 35.83±9.18MPa; yield stress 25.37±3.57MPa) while formulation 3 is the weakest (Young's modulus 28.47±4.94MPa; ultimate strength 1.18±0.23MPa; yield stress 0.73±0.16MPa). The extensibility of the fibres is described by the ultimate strain value (Figure 4.15) demonstrating a distinctly different distribution compared to Young's modulus, ultimate strength and yield stress charts (Figure 4.12-4.14). Formulation 7, demonstrating high tensile strength had the lowest ultimate strain (0.08±0.05). The most extensible formulation was formulation 10 with the highest ultimate strain value (0.61±0.11).

The formulation components, plasticiser, crosslinker and polymer concentration are noted in literature to affect the tensile properties of the matrix. Kuo and Ma (2007) reported that crosslinker density and concentration as well as polymer concentration affected the mechanical properties the crosslinked alginate matrix. Crosslinked alginate matrices once formed are brittle, but incorporation of a plasticiser reduces the interaction between polymer chains resulting in a flexible structure (da Silva *et al*., 2009). Plasticised crosslinked alginate films result in reduced tensile strength while increased elongation of the matrix (Olivas and Barbosa-Cànovas, 2008; da Silva *et al*., 2009). To elucidate the extent to which the fibre matrix is influenced by the concentration of alginate, barium chloride 2-hydrate and glycerol, Young's modulus and ultimate strain were identified as responses for optimisation.

Table 4.3: Cross-sectional area of the experimental design formulations (N=5)

Experimental design formulation	*Cross-sectional area (mm^2)*
1	0.07±0.03
2	0.20±0.05
3	0.11±0.02
4	0.14±0.03
5	0.17±0.05
6	0.21±0.07
7	0.05±0.01
8	0.14±0.03
9	0.09±0.04
10	0.12±0.02
11	0.09±0.02
12	0.14±0.03
13	0.27±0.01
14	0.05±0.01
15	0.17±0.05

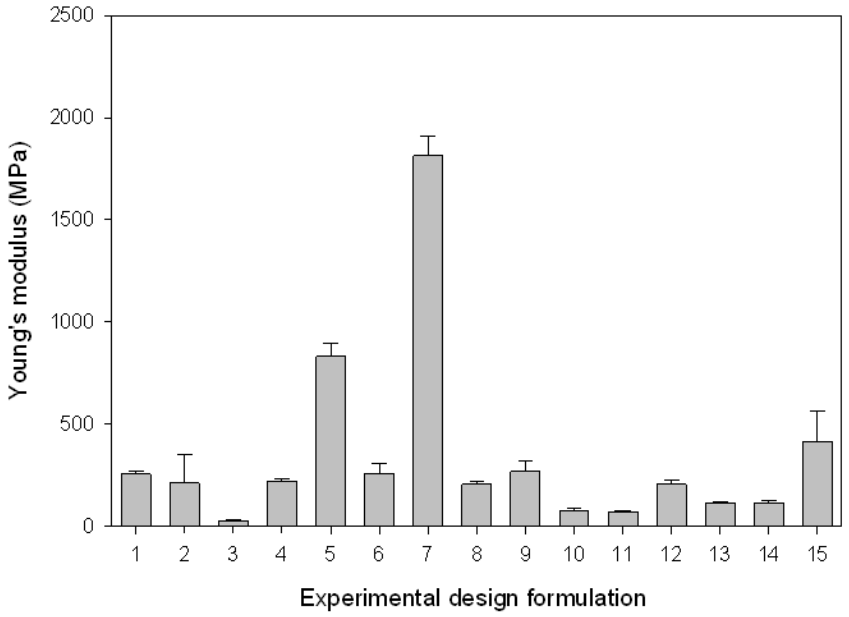


Figure 4.12: Vertical bar chart of Young's Modulus values for the 15 experimental design formulations (N=5)

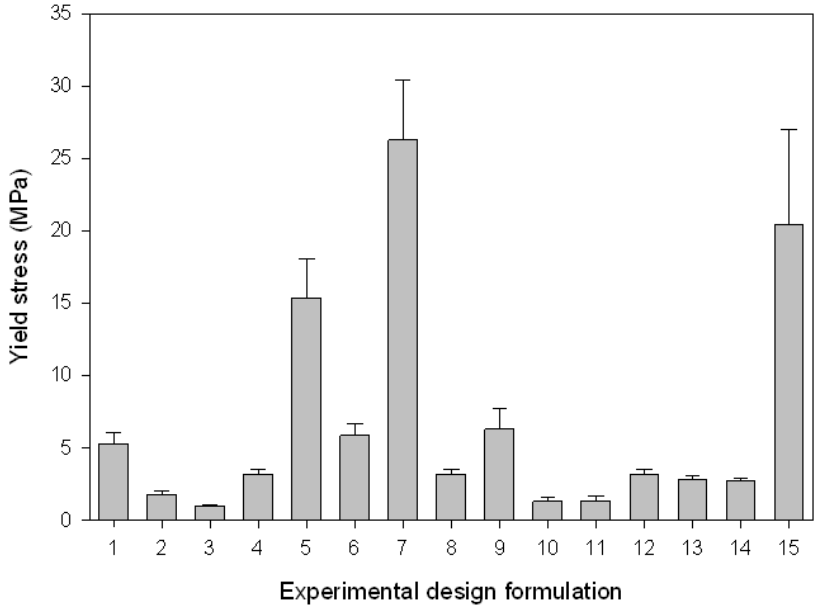


Figure 4.13: Vertical bar chart of yield stress values for the 15 experimental design formulations (N=5)

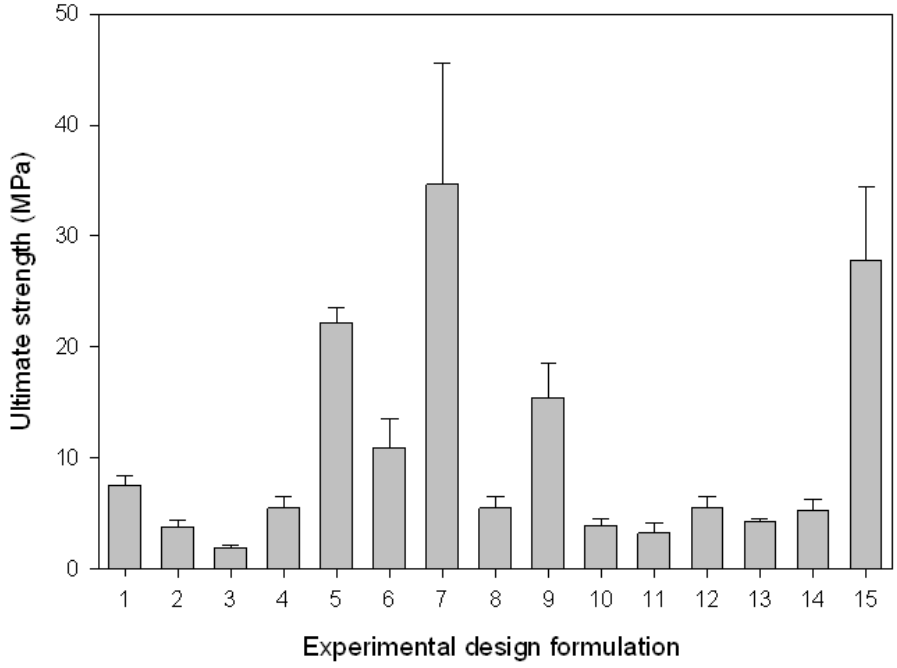


Figure 4.14: Vertical bar chart of ultimate strength values for the 15 experimental design formulations (N=5)

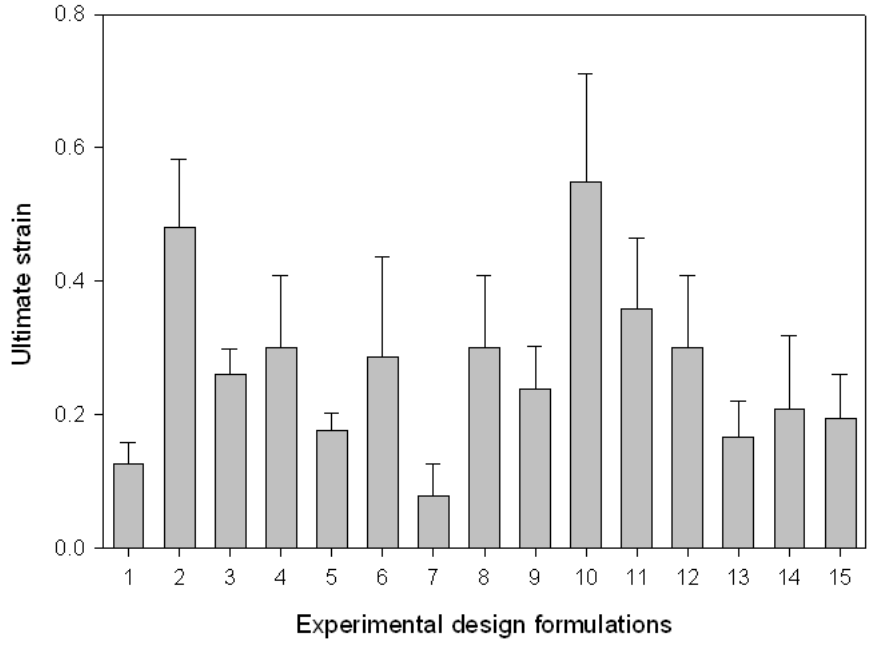


Figure 4.15: Vertical bar chart of ultimate strain values for the 15 experimental design formulations (N=5)

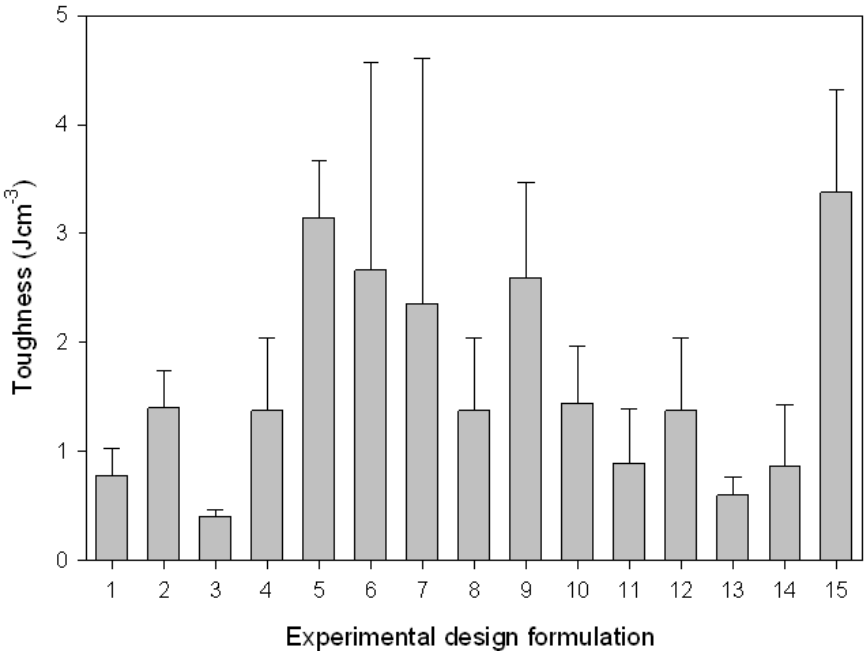
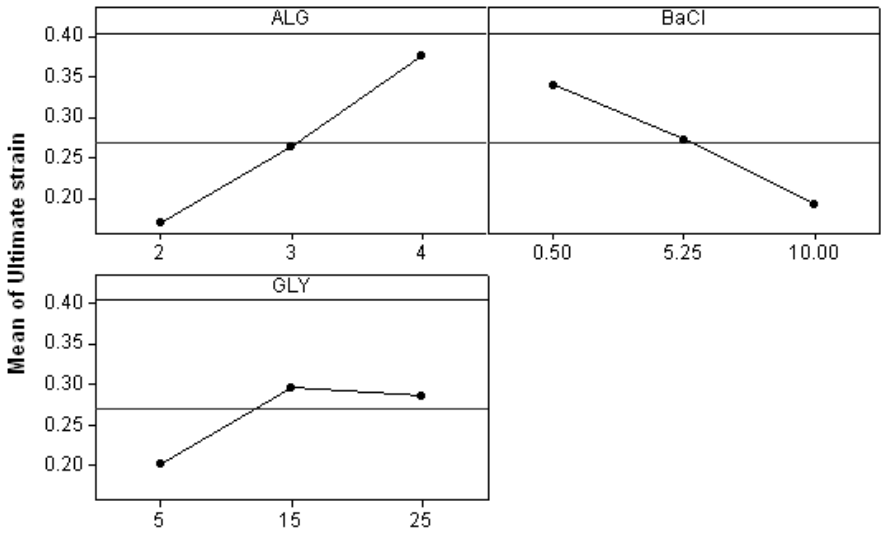


Figure 4.16: Vertical bar chart of toughness values for the 15 experimental design formulations (N=5)

The Young's modulus and ultimate strain values were assessed in optimisation of the experimental design formulations. Main effect plots related the formulation components, alginate, glycerol and barium chloride concentrations to the ultimate strain and Young's modulus, represented in Figure 17a and 18a respectively. A linear relationship is observed between ultimate strain and alginate concentration (Figure 4.17a), increasing the polymer concentration therefore resulted in fibres that could withstand a higher strain. On the contrary as the amount of barium chloride increased, the ultimate strain decreased and resulted in rigid fibres (Figure 4.17a). Increasing the quantity of the plasticiser, glycerol, initially increased the strain, though raising the quantity above 15mL had little effect (Figure 4.17a). Young's modulus values were higher at lower alginate and glycerol concentrations and higher barium chloride concentrations. The plasticiser, glycerol, has the greatest effect on the Young's modulus (Figure 4.18a). The mean Young's modulus value is greatest, approximately 800MPa, when the lowest quantity of glycerol is added. This value plummets to below 200MPa as the quantity of glycerol added increases to 15mL.

The interaction between two formulation components and either ultimate strain or Young's modulus are apparent in Figure 17b and 18b correspondingly. Interactions between alginate and barium chloride as well as glycerol and alginate were significant with respect to ultimate strain values. Rigid fibres were formulated when the concentration barium chloride was high and the alginate concentration is low as well as when concentration of glycerol was low and the alginate concentration was high (Figure 4.17b). The most flexible fibres were formulated using a higher alginate concentrations combined with either lower barium chloride concentrations or higher quantities of glycerol (Figure 4.17b). An interesting relationship was found to exist between the formulations components and the Young's modulus values. The highest Young's modulus value was attained when the lowest volume of glycerol was combined with a $2\%^{w}/_{v}$ alginate solution (Figure 4.18b). The interaction between crosslinking salt and plasticiser was noteworthy at higher concentrations of barium chloride with the lowest quantity of glycerol added (Figure 4.18b).

(a)



(b)

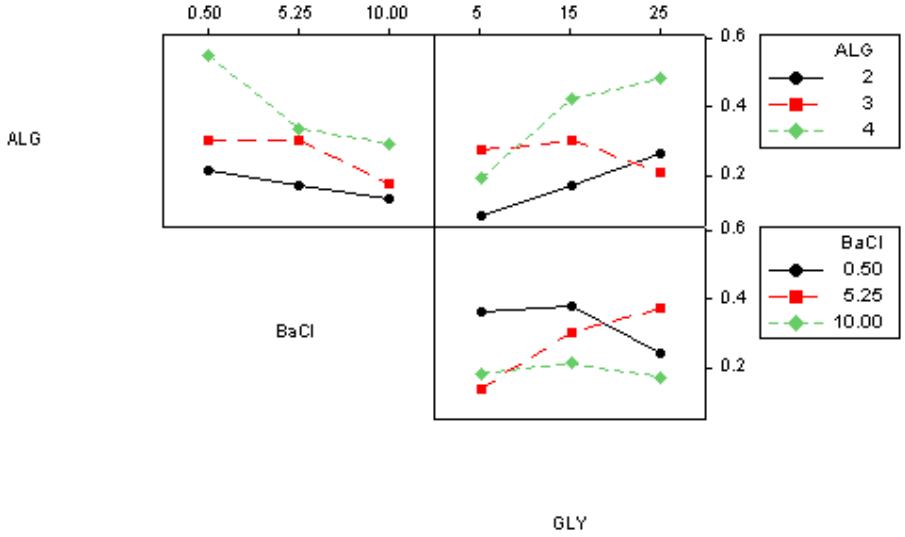
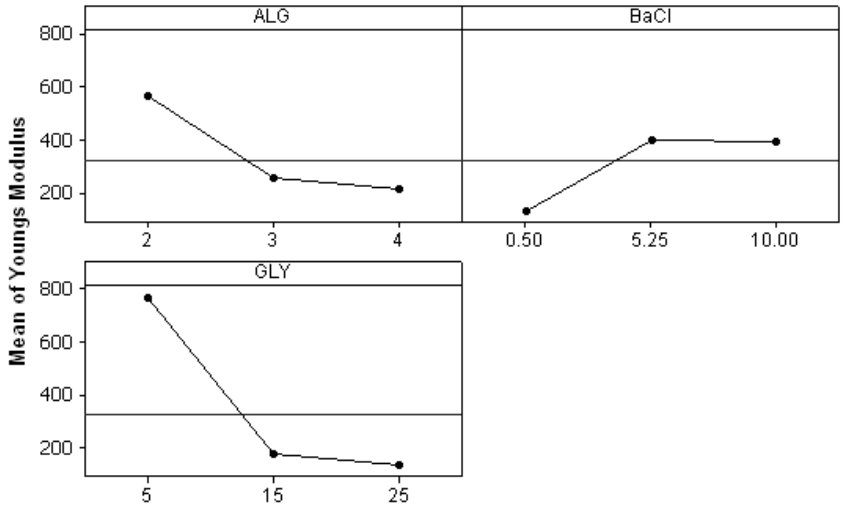


Figure 4.17: Factor plots, (a) main effects plot and (b) interaction plots, depicting the correlation between ultimate strain and design variables

(a)



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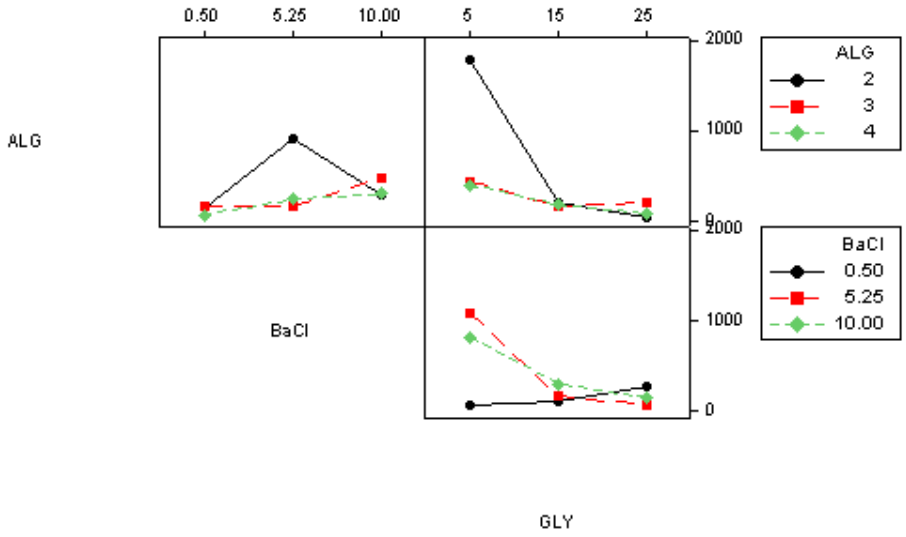


Figure 4.18: Factor plots, (a) main effects plot and (b) interaction plots, depicting the correlation between Young's modulus and design variables

4.4.5. Analysis of the Box-Behnken response surface design

Data used for the optimisation of experimental design formulations related to drug release in PBS pH 6.8 as well as tensile analysis to determine the strength and flexibility of fibres, therefore the MDT over 10 days for ciprofloxacin and diclofenac were measured in addition to calculating the Young's modulus and ultimate strain values. Experimental and predicted values for the responses are reviewed in Table 4.4.

The residual plots (Figure 4.19) for $MDT_{10\ days}$ for ciprofloxacin and diclofenac, Young's modulus and ultimate strain were employed for analysis of the model. Analysis of the normal plot of the residuals shows normal distribution as all points are in close proximity to the straight line (Figure 4.19a-d). One outlying point is noted on the $MDT_{10\ days\ diclo}$ curve (Figure 4.19b). Even distribution on either side of the zero line is evident in the fitted residual versus fitted value plots (Figure 4.19e-f). A bell-shaped histogram indicates normal distribution of data (Figure 4.19k-l). Slightly irregular histograms are apparent for $MDT_{10\ days}$ for both diclofenac and ciprofloxacin release (Figure 4.19i-j). Regular fluctuation on either side of the centre line displayed in the residual versus observation order plots suggest the error terms are not correlated with one another (Figure 4.19m-p).

The coefficient of determination assesses the extent that the model fits the data, the R^2 for Young's modulus, ultimate strain and $MDT_{10days\ diclo}$ were above 75%, however, the $MDT_{10days\ cipro}$ is significantly lower at 56.2%.

The regression equations generated for $MDT_{10\ days}$ for diclofenac and ciprofloxacin, Young's modulus and ultimate strain are specified in Equations 4.2-4.5:

$$MDT_{10daysdiclo}=13.9262+(7.0651)[ALG]+2.6392[BaCl]+0.1087[GLY]+1.9542[ALG*ALG]+(0.1272)[BaCl*BaCl]+0.0008[GLY*GLY]+(-0.1947)[ALG*BaCl]+(-0.0690)[ALG*GLY]+(0.0075)[BaCl*GLY]$$

Equation 4.2

$$MDT_{10dayscipro}=23.2545+(7.4112)[ALG]+1.5069[BaCl]+(0.8573)[GLY]+1.1508[ALG*ALG]+(0.0502)[BaCl*BaCl]+0.0021[GLY*GLY]+(-0.6100)[ALG*BaCl]+0.2615[ALG*GLY]+0.0673[BaCl*GLY]$$

Equation 4.3

$$Young'smodulus=4117.20+(1604.69)[ALG]+133.22[BaCl]+(196.17)[GLY]+145.24[ALG*ALG]+(4.71)[BaCl*BaCl]+2.73[GLY*GLY]+4.33[ALG*BaCl]+34.64[ALG*GLY]+(-4.65)[BaCl*GLY]$$

Equation 4.4

$$Ultimatestrain=(0.058696)+0.082237[ALG]+0.008864[BaCl]+0.008711[GLY]+0.005000[ALG*ALG]+(0.000443)[BaCl*BaCl]+(0.000525)[GLY*GLY]+(0.009474)[ALG*BaCl]+0.002750[ALG*GLY]+0.000579[BaCl*GLY]$$

Equation 4.5

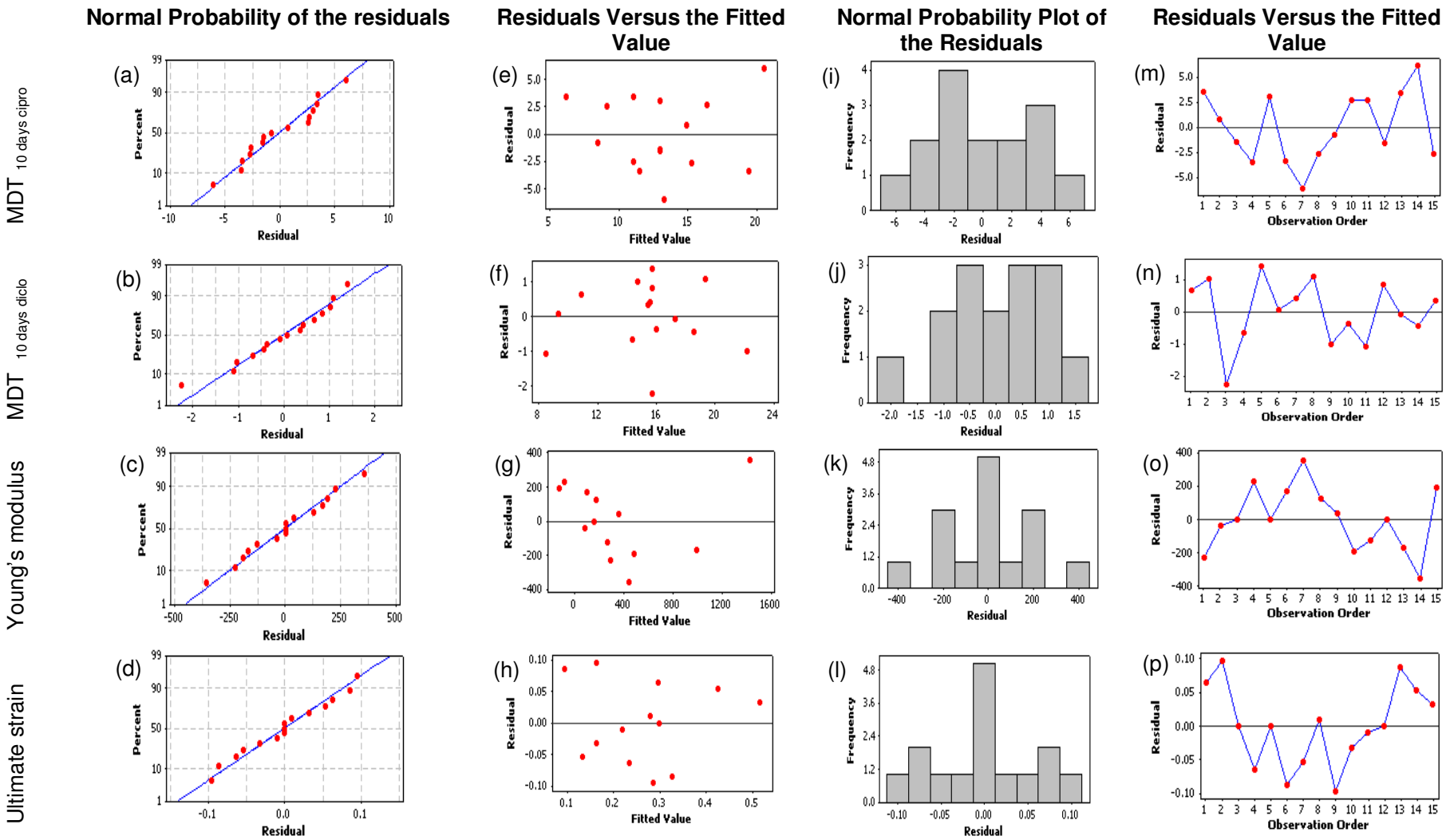


Figure 4.19: Summary of residual plots for $MDT_{10\ days}$ for the release of diclofenac sodium and ciprofloxacin, Young's modulus and ultimate strain

diclo

Table 4.4: Correlation between experimental and predicted values for Young's modulus, ultimate strain and $MDT_{10\ days}$ for the release of diclofenac sodium and ciprofloxacin

	Young's Modulus (MPa)			*Ultimate strain*			*MDT 10 days Diclo*			*MDT 10 days Cipro*		
Formulation	*Experimental*	*Predicted*	*St residual*	*Experimental*	*Predicted*	*St residual*	*Experimental*	*Predicted*	*St residual*	*Experimental*	*Predicted*	*St residual*
1	290.95	481.06	-1.17	0.13	0.162	-0.65	15.66	16.02	-0.43	19.12	16.45	0.93
2	81.32	439.56	-2.21	0.48	0.426	1.08	18.13	18.56	-0.51	26.71	20.61	2.12
3	38.55	78.53	-0.25	0.26	0.164	1.93	15.75	14.73	1.23	15.75	14.96	0.28
4	157.94	157.94	0.00	0.30	0.30	0.00	13.48	13.48	-1.66	11.52	12.98	-0.31
5	823.31	991.44	1.04	0.18	0.094	1.73	17.23	17.30	-0.08	9.61	6.19	1.19
6	298.52	170.38	0.79	0.29	0.28	0.2	20.46	19.37	1.32	8.45	11.08	-0.91
7	1784.00	1425.77	2.21	0.08	0.134	-1.08	16.02	15.60	0.51	7.21	13.31	-2.12
8	157.94	157.94	0.00	0.30	0.30	0.00	17.13	15.73	1.04	16.04	12.98	0.65
9	267.71	99.58	1.04	0.24	0.326	-1.73	9.36	9.29	0.08	8.12	11.54	-1.19
10	61.91	-128.202	1.17	0.55	0.518	0.65	15.81	15.46	0.43	12.68	15.34	-0.93
11	62.51	292.60	-1.42	0.36	0.27	1.28	11.49	10.83	0.80	14.53	11.06	1.21
12	157.94	157.94	0.00	0.30	0.30	0.00	16.57	15.73	0.62	11.39	12.98	-0.34
13	145.75	-84.34	1.42	0.17	0.234	-1.28	13.68	14.35	0.80	15.99	19.46	-1.21
14	136.6	264.74	-0.79	0.21	0.22	-0.20	7.31	8.40	-1.32	11.76	9.13	0.91
15	401.9	361.11	0.25	0.19	0.29	-1.93	21.16	22.18	-1.23	7.71	8.51	-0.28

A p-value under 0.05, highlighted in Table 4.5, indicates a significant relationship between the factor and the response which is identified in Table 4.5. The relationships worth noting are the glycerol concentration (p=0.044) and its effect on the Young's modulus as well as the effect of barium chloride concentration (p=0.14, BaCl; p=0.021 BaCl*BaCl) on $MDT_{10\ days}$ of diclofenac.

Table 4.5: Probability of the test (p-test) obtaining the measured responses, highlighted are the significant values (<0.05)

Term	*p-value*			
	Young's modulus	*Ultimate strain*	*MDT 10 days diclofenac*	*MDT 10 days ciprofloxacin*
Constant	0.073	0.920	0.193	0.502
ALG	0.192	0.811	0.249	0.710
BaCl	0.389	0.846	0.014	0.573
GLY	0.044	0.715	0.783	0.539
ALG*ALG	0.429	0.927	0.073	0.716
BaCl*BaCl	0.557	0.855	0.021	0.721
GLY*GLY	0.167	0.358	0.926	0.947
ALG*BaCl	0.904	0.408	0.314	0.360
ALG*GLY	0.080	0.605	0.442	0.405
BaCl*GLY	0.232	0.605	0.686	0.317

4.4.6. Response surface analysis

Response surface methodology is defined by Myers *et al.* (2004) as "a collection of statistical and optimisation techniques used to optimise processes and product designs". Response surface methods were employed to observe the relationship that exists between the three factors of polymer, plasticiser and crosslinking salt concentrations. MDT for ciprofloxacin and diclofenac (PBS, pH 6.8) over ten days, the strain the fibres could withstand without fracturing and Young's modulus were the variables which the factors were analysed against. The surface plots, shown in Figures 4.20, 4.22 and 4.24, provide valuable information regarding the interaction between two factors and a response.

4.4.6.1. Response surface analysis for $MDT_{10\ days}$ of ciprofloxacin and diclofenac (PBS pH 6.8)

Analysis of the response surface plots provided information regarding interaction between two variables on the MDT for the release of either ciprofloxacin or diclofenac (Figure 4.20). Interaction between the polymer, crosslinking salt and plasticiser in the formation of a fibre was found to have a definite effect on the drug release from the matrix. The effect of drug solubility discussed in Section 4.4.3 influences the dissolution data. Further considerations are however necessary in the assessment of drug release. Alginate is pH responsive polymer, therefore the crosslinked alginate fibres initially swell and then erode when submerged in PBS pH 6.8.

The complexity of the relationship existing between the $MDT_{10\ days}$ for the release of ciprofloaxin together with the alginate and barium chloride concentrations was depicted in Figure 4.20a. To visualise this relationship more clearly a contour plot was assessed (Figure 4.21). At low polymer and crosslinking salt concentrations the MDT was the lowest. However, the highest MDT was recorded when the 2%$^{w}/_{v}$ alginate solution was crosslinked in a 10%$^{w}/_{v}$ barium chloride solution as well as when a 4%$^{w}/_{v}$ alginate solution was crosslinked in 0.5%$^{w}/_{v}$ barium chloride solution. $MDT_{10\ days}$ for the diclofenac release (Figure 4.20d) was maximised when a 4%$^{w}/_{v}$ alginate solution was crosslinked in a barium chloride solution where the concentration exceeded 5%$^{w}/_{v}$.

The inclusion of a plasticiser in the formulation was expected to disrupt the interaction between polymer and cations present in solution. Addition of a plasticiser considerably increased $MDT_{10\ days}$ for the release of ciprofloxacin as depicted Figure 4.20b and c. Glycerol had a less prominent effect on the diclofenac drug release (Figure 4.20e and f).

Gelation, induced by the binding of the barium cations with the polymeric structure, ultimately had the greatest affect on $MDT_{10\ days}$ for diclofenac release ($p=0.014$). MDT steadily increased with increasing concentrations of barium chloride (Figure 4.20d and f).

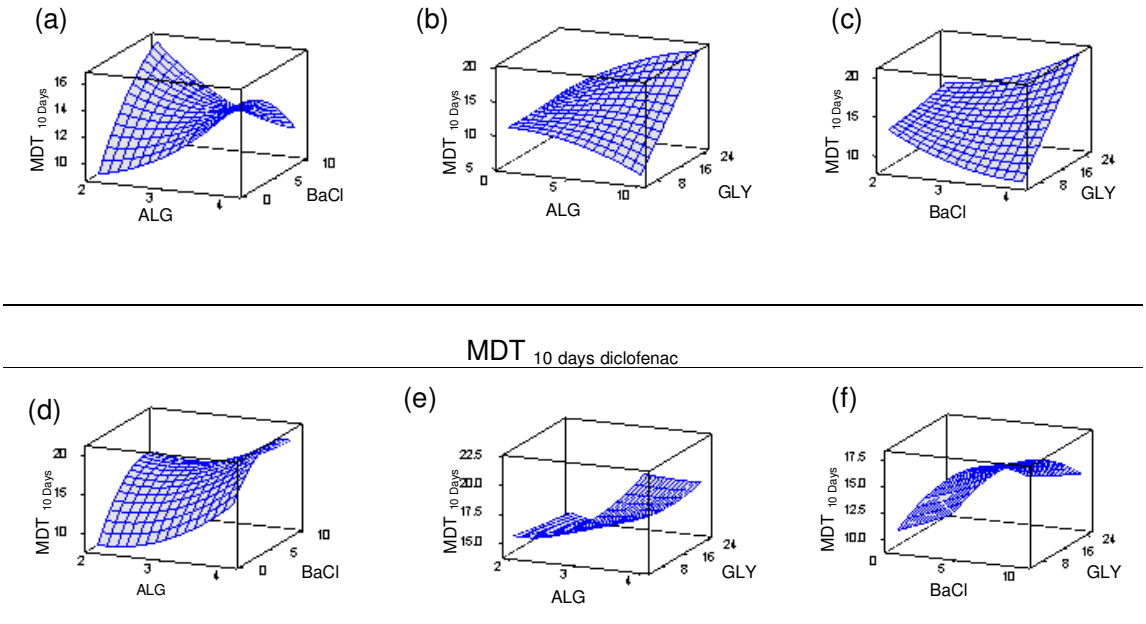


Figure 4.20: Response surface plots relating $MDT_{10\ days}$ for the release of ciprofloxacin to design variables (a) alginate (ALG) and barium chloride (BaCl), (b) alginate (ALG) and glycerol (GLY) and (c) barium chloride (BaCl) and glycerol (GLY) as well as comparing $MDT_{10\ days}$ for the release of diclofenac to design variables (d) alginate (ALG) and barium chloride (BaCl), (e) alginate (ALG) and glycerol (GLY) and (f) barium chloride (BaCl) and glycerol (GLY)

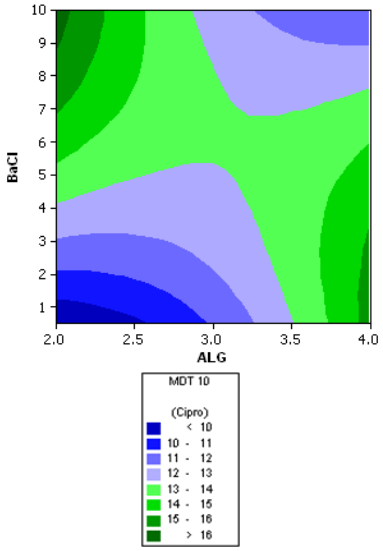


Figure 4.21: Contour plot depicting the relationship between $MDT_{10\ days}$ for the release of ciprofloxacin and the concentration formulation variables, barium chloride (BaCl) and alginate (ALG). Hold value for glycerol 15mL

4.4.6.2. Response surface analysis for Young's modulus and ultimate strain

Barium crosslinked alginate fibres formed a rigid matrix that would not fit within the periodontal pocket without fracturing of the fibre. Addition of a plasticiser reduced the extent to which cations bind to form the crosslinked matrix and resulted in elastic fibres that were easily manipulated for placement in the periodontal pocket while retaining their form. To understand the relationship between the variables and reponses on the strength and flexibility of the fibre, surface and contour plots (Figure 4.22-4.25) were assessed.

Glycerol had the most significant effect on the Young's modulus (p=0.044). Following analysis of surface plots (Figure 4.22b and c) and contour plots (Figure 4.23a and b), the relationship between glycerol with the variables and Young's modulus were further explained. After the subsequent addition of plasticiser, the fibres became elastic and deformed under the applied stress until finally fracturing. When more than 10mL of glycerol was added the fibres weakened as the Young's modulus values noticeably dropped below 200MPa.

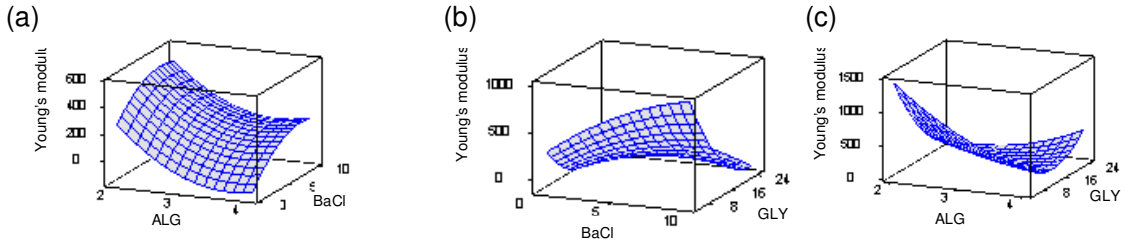


Figure 4.22: Response surface plots relating Young's modulus to design variables (a) alginate (ALG) and barium chloride (BaCl), (b) alginate (ALG) and glycerol (GLY) and (c) barium chloride (BaCl) and glycerol (GLY)

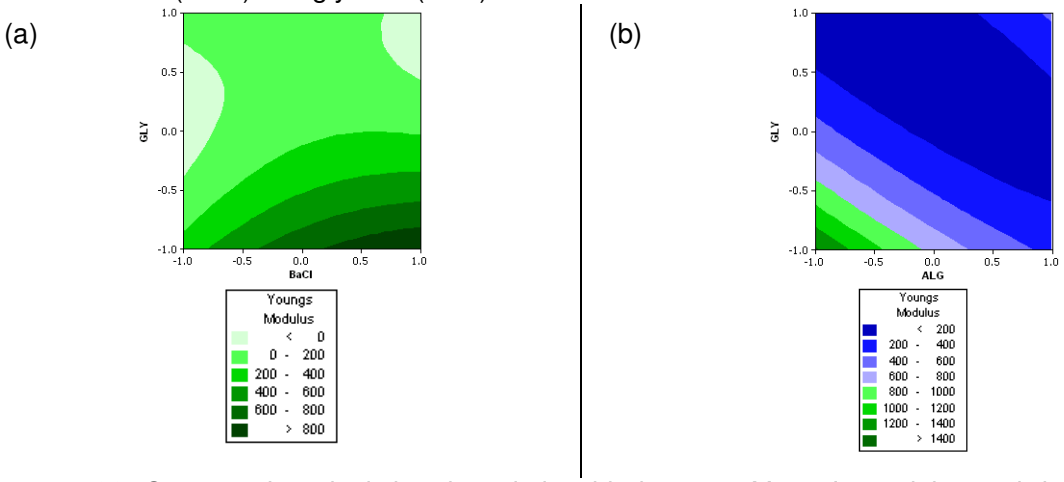


Figure 4.23: Contour plots depicting the relationship between Young's modulus and design variables (a) barium chloride (BaCl) and glycerol (GLY) (hold value for alginate 3%$^{w}/_{v}$) and (b) glycerol (GLY) and alginate (ALG) (hold value for barium chloride 5.25%$^{w}/_{v}$)

The effect of the variables on the extensibility of the fibres is depicted in Figure 4.24 and 4.25. The effect of increasing concentrations of cations present in solution had a negative result on strain and formed in a brittle matrix. This is evident in Figure 4.24a and b as a drop in strain values occurred with increased concentrations of barium chloride ions. To describe the relationship between glycerol and alginate on the strain the fibre endured the contour plot (Figure 4.25) was assessed. A rise in glycerol and alginate quantities caused an increase in the degree of deformation the matrix endured and higher ultimate strain values were reached before fracture.

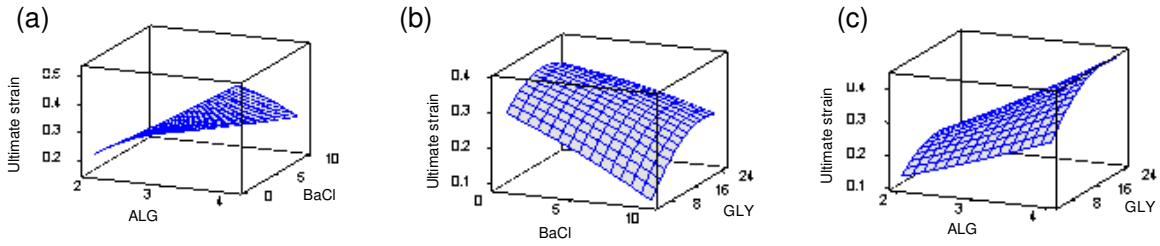


Figure 4.24: Response surface plots relating ultimate strain to design variables (a) alginate (ALG) and barium chloride (BaCl), (b) alginate (ALG) and glycerol (GLY) and (c) barium chloride (BaCl) and glycerol (GLY)

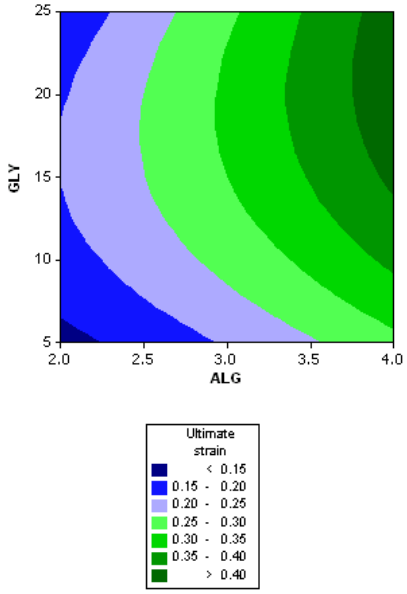


Figure 4.25: Contour plot depicting the change in ultimate strain with varying glycerol (GLY) and alginate (ALG) concentrations. Hold value for barium chloride 5.25%$^{w}/_{v}$

To establish a relationship between Young's modulus, ultimate strain and formulation variables overlaid contour plots were generated. The Young's modulus and ultimate strain ranges were set between 100-300MPa and 0.2-0.4 respectively. The opaque regions in Figure 4.26 represented the concentrations of the variables which according to data would produce formulations fitting within these parameters. In Figure 4.26 it is evident that the variables, Young's modulus and ultimate strain are inter-related.

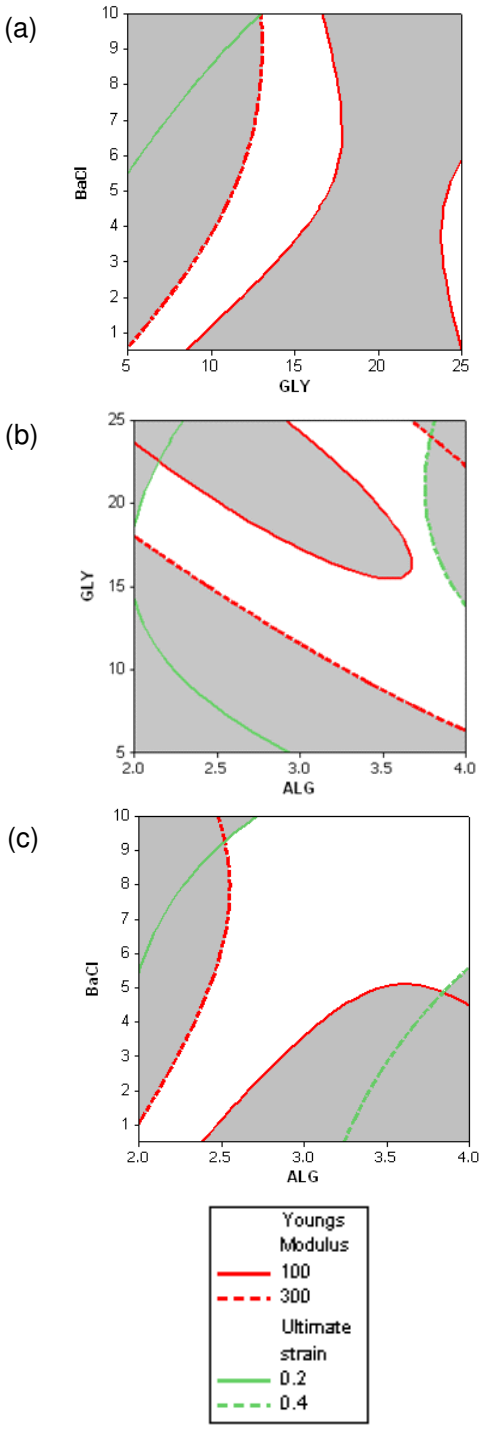


Figure 4.26: Overlaid plots establishing a relationship between Young's modulus, ultimate strain and design variables: (a) barium chloride (BaCl) and glycerol (GLY) (hold value for alginate 2%$^{w}/_{v}$), (b) glycerol (GLY) and alginate (ALG) (hold value for barium chloride 5.25%$^{w}/_{v}$) and (c) barium chloride (BaCl) and alginate (ALG) (hold value for glycerol 15mL)

4.4.7. Response optimisation

Fibre optimisation was conducted using the measured responses for $MDT_{10\ days}$ for the release of ciprofloxacin and diclofenac as well as the Young's modulus and ultimate strain. The aim was to show that the optimised levels of variables would result in strong yet flexible fibres releasing at the drug at the desired rate, resulting in favourable MDT for ciprofloxacin and diclofenac over 10 days. Optimisation plots, Figure 4.27 and 4.28 for two optimal formulations were developed with a composite desirability for formulation 1 of 91.00% and formulation 2 of 88.90%.

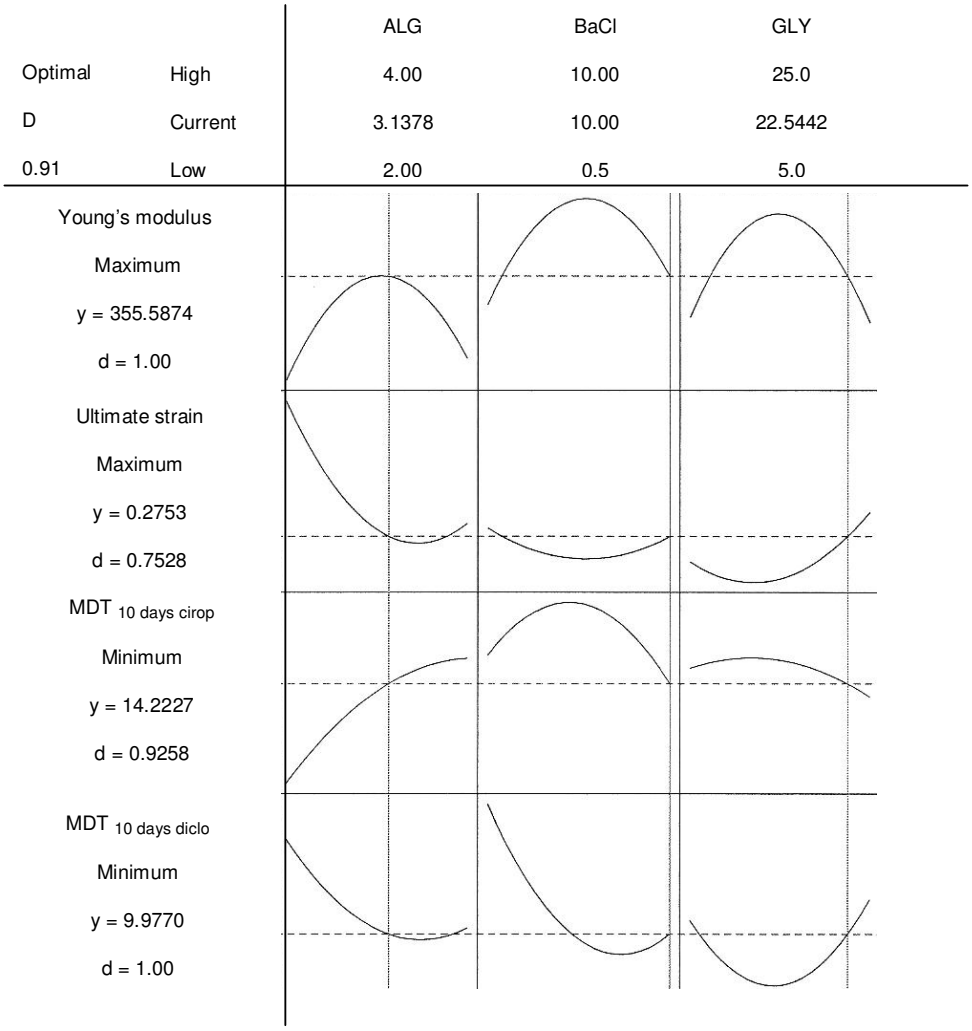


Figure 4.27: Optimised plots for formulation 1 for the optimisation of the PFD

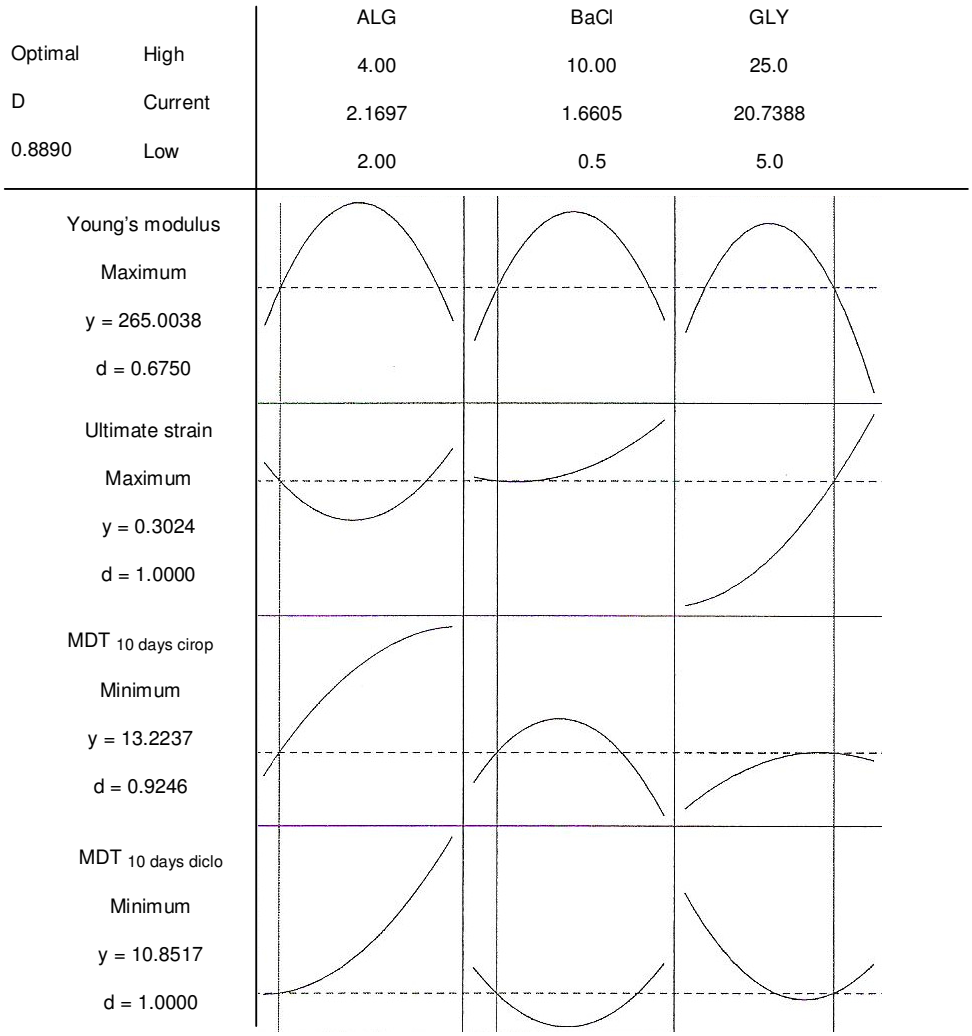


Figure 4.28: Optimised plots for formulation 2 for the optimisation the PFD

Ideal formulations were prepared instituting the optimal setting. The experimental value was compared to the predicted value as shown in Table 4.6. The Young's modulus, ultimate strain and $MDT_{10\ days}$ for formulation 1 were similar to the expected values (R^2 = 0.998) therefore the formulation was in near agreement to what was predicted. The only response in formulation 2 that correlated with the predicted value was ultimate strain, resulting in a less than perfect fit (R^2 = 0.942). The analysis the MDT demonstrated greater deviation from predicted values compared to other variables. Therefore, a more sensitive and accurate method of detecting drug release was developed based on liquid chromatography principles (Chapter 5).

Table 4.6: Experimental and predicted values for optimised formulation 1 and 2

Response	*Formulation 1*			*Formulation 2*		
	Predicted Value	*Experimental Value*	*Desirability (%)*	*Predicted Value*	*Experimental Value*	*Desirability (%)*
Youngs modulus	355.60MPa	314.04MPa	100.00	265.00MPa	81.92MPa	67.50
Ultimate strain	0.27	0.29	75.28	0.30	0.29	100.00
MDT 10 days – ciprofloxacin	14.22	14.77	92.58	13.22	20.63	92.55
MDT 10 days – diclofenac	9.98	28.19	100.00	10.85	31.27	100.00

4.5. Concluding Remarks

Preliminary investigations identified the polymer, crosslinking salt and plasticiser concentration as factors upon which the overall physicochemical and physiomechanical behaviour of the fibre formulation was dependent upon. Upper and lower levels for each factor were determined experimentally and employed in the generation of a 3-factor Box-Behnken Design. The DoE created 15 random formulations that were assessed using drug entrapment, *in vitro* drug release and tensile strength as the possible responses. Four responses were further identified for optimisation: $MDT_{10\ days}$ of ciprofloxacin and diclofenac sodium, Young's modulus and ultimate strain. The design was evaluated according to the extent to which it fitted the predicted responses using standardised residuals, p-values and coefficients of determination as well as residual and factor plots. Analysis revealed that the alginate and glycerol composition had a notable effect on mechanical properties of the matrix while the binding of barium ions to the polymer complex had a considerable result on drug release behaviour.

Constrained settings for optimisation were ascertained and two optimised formulations were prepared and tested against optimisation responses. The experimental values for Young's modulus and ultimate strain tests were similar to the predicted values. However, the drug release studies did not fit the model as well as predicted. The responses for formulation 1 were in close agreement to the predicted values and formulation 1 was used in subsequent studies for further analysis. This confirmed that the Design of Experiment is a suitable means for optimising the PFD.

CHAPTER 5

EVALUATION OF THE PHYSICOCHEMICAL AND PHYSICOMECHANICAL PROPERTIES OF AN OPTIMISED CIPROFLOXACIN- AND DICLOFENAC-LOADED POLYMERIC FIBRE DEVICE

5.1. Introduction

The challenge facing pharmaceutical research is to ensure that the *in vitro* activity of a product leads to sufficiently high concentrations of drug present at the site of action to ensure a therapeutic response (Tønnesen and Karlsen, 2002). This in turn, has lead to the development of targeted drug delivery devices, yet many of these fail to meet the necessary requirements. During the 1990's numerous antimicrobial-loaded devices for the treatment of PD were developed, however, most of these had limited success (Chapple, 2009). Therefore providing was the rationale for developing a device capable of simultaneously delivering two drugs, an antimicrobial and anti-inflammatory agent, over an extended period of time within the periodontal pocket.

Local delivery systems placed within the periodontal pocket needs to meet specific criteria. The formulation should be both biocompatible and biodegradable delivering the chemotherapeutic agent(s) over the intended period of time. As well as being easily inserted within the periodontal pocket, remain in place for the duration of treatment and release sufficient drug to adequately meet the therapeutic levels required. These criteria necessitate specific chemical and mechanical *in vitro* analysis of the device. Drury *et al.* (2004) stated when reviewing alginate hydrogels that the "…knowledge of the mechanical and material properties of the hydrogels and the properties that govern and influence them is necessary to adequately design and effectively use these systems." Likewise, it is necessary to assess the *in vitro* behaviour of the alginate based fibre in forecasting its success *in vivo*. The optimisation of the PFD described in Chapter 4 yielded a prototype fibre. Further investigation predicted the usefulness of such a device in the treatment of PD.

5.2. Materials

Double deionised water was obtained from a Milli-Q system (Milli-Q, Millipore Johannesburg). UPLC grade acetonitrile and methanol were purchased from Romil Pure Chemistry, distributed by Microsep, Johannesburg. Formic acid (99%) was purchased from Merck, Wadeville. Sample vials (2mL pre-silt, PTFE/silicone septa, screw top) and the Aquity UPLC® Bridged Ethyl Hybrid (BEH) Shield RP_{18} column was procured from Waters, USA. All further chemicals were purchased from suppliers as outlined in previous chapters.

5.3. Methods

5.3.1. Preparation of optimised fibres

The optimised PFD described in Chapter 4 yielded a formula which employed set polymer, crosslinking salt and plasticiser combinations as indicated in Table 5.1. This formulation was prepared as (1) ciprofloxacin-loaded (200mg), (2) diclofenac sodium-loaded (500mg), (3) ciprofloxacin(200mg)/diclofenac sodium(500mg)-loaded fibres and (4) drug-free. The fibres were prepared as outlined in Chapter 3 Section 3.3.1. with the model drugs dispersed in water prior to addition of alginate.

Table 5.1: Formulation components for optimised fibres prepared

Formulation components	*Formulation number*			
	1	*2*	*3*	*4*
Alginate (%$^{w}/_{v}$)	3.14	3.14	3.14	3.14
Glycerol (mL)	22.54	22.54	22.54	22.54
Barium chloride 2-hydrate (%$^{w}/_{v}$)	10.00	10.00	10.00	10.00
Ciprofloxacin (mg)	200	-	200	-
Diclofenac sodium (mg)	-	500	500	-

5.3.2. Characterisation of morphological transitions

Samples were prepared and analysed as outlined in Chapter 3 Section 3.3.2.

5.3.3. Development of a method for the co-detection of ciprofloxacin and diclofenac sodium

Ciprofloxacin and diclofenac sodium absorb UV light within the same range, λ_{max}=278nm and λ_{max}=276 nm respectively. A method to simultaneously detect the model drugs was needed. An ultraperformance liquid chromatography (UPLC) method was developed using a Waters® Aquity Performance LC system (Waters, Milford, MA, USA) coupled with a photodiode array detector (PDA). The UPLC was fitted with an Aquity UPLC® Bridged Ethyl Hybrid (BEH) Shield RP_{18} column, with a pore size of 1.7µm. A gradient method with a run time of 3 minutes, depicted in Table 5.2, was developed using acetonitrile and 0.1%$^{v}/_{v}$ formic acid in double deionised water as the mobile phases. Ciprofloxacin and diclofenac samples were filtered, using 0.22µm syringe filter, into 2mL vials. The vials were placed in the UPLC sample holder. The injection volume was standardised at 2µL per injection.

Table 5.2: Gradient method used in the separation of ciprofloxacin and diclofenac sodium indicating mobile phase concentrations and flow rate at various time periods over the 3 minute run time

Time (min)	*Flow rate (mL/min)*	*0.1 %$^{v}/_{v}$ Formic acid solution*	*Acetonitrile*
Initial	0.5	85%	15%
1.00	0.5	20%	80%
3.00	0.5	85%	15%

5.3.4. Determination of drug entrapment of optimised fibres

Using the UPLC method developed, determining the quantity of the two model drugs entrapped within a fibre was possible. Model drugs, ciprofloxacin and diclofenac sodium, were dissolved in PBS pH 8 to generated standard calibration curves for subsequent determination of the drug entrapped in the optimised fibre. Serial injections (2µL) of varying concentrations of drug were injected and the area under the curve (AUC) was calculated for peaks at 0.8 and 1.6 minutes for ciprofloxacin and diclofenac sodium respectively. This was plotted against drug concentration and a calibration curve created.

The addition of glycerol to crosslinked barium fibres generated a strong yet flexible matrix which presented difficulties in milling and dissolving the formulation, which is necessary to quantify the amount of drug entrapped. Approximately 25mg of fibres were submerged in 100mL PBS pH 8 and stirred until the fibres had disintegrated. The samples were then filtered through a 0.22µm filter into a 2mL sample vial. A 2µL sample was injected and UV absorbance detected at λ=278nm. The area under the curve was calculated and compared to the calibration curve to determine the concentration of model drug entrapped within the fibres.

5.3.5. Determination of drug release from optimised fibres

Ciprofloxacin- and diclofenac-loaded fibres were assessed for the simultaneous release of these two drugs. The dissolution apparatus used was the same as described in Chapter 3 Section 3.3.6. The drug release studies were conducted in triplicate. Samples were removed on days 0, 1, 2, 4, 6, 8 10 and frozen at -7˚C until analysis. Samples were thawed at room temperature and filtered using a 0.22µm syringe filter into a 2mL sample vial. Once all the samples were filtered and loaded, a sample set was run and the autosampler injected 2µL of each sample according to the specified method (Section 5.3.3). Each sample was tested in triplicate. Standard samples were run after the dissolution samples had been tested.

For comparison standard samples contained a known quantity of ciprofloxacin and diclofenac sodium dissolved in a 50:50 solution of doubled deionised water and acetonitrile. Serial dilutions with varying concentrations of drugs were injected. The areas under the curve for the relevant peaks were calculated and plotted against concentration of drug to construct a calibration curve, against which the dissolution samples were compared.

5.3.6. Characterisation of textural properties

Textural analysis on drug-loaded and drug-free fibres would elucidate the effect of the model drugs on the strength and flexibility of the ciprofloxacin-, diclofenac- and ciprofloxacin/diclofenac-loaded fibres to the drug-free formulation. The same method as described in Chapter 3 Section 3.3.7.3 was used.

5.3.7. Characterisation of hydration dynamics behaviour

Once fibres were submerged in PBS the matrices displayed diverse behaviour depending on the pH of the solution. To investigate the hydration behaviour of the optimised formulation, drug-free and drug-loaded fibres were separately assessed in 20mL of PBS pH 4 or pH 6.8 and placed in a shaking incubator (Orbital Shaker Incubator, LM-530, Lasec Scientific Equipment, Johannesburg, South Africa) for 10 days. Samples were removed on day 0, 1, 2, 4, 6, 8 and 10. Once removed the degree of swelling and erosion was determined through measuring the diameter of the fibre. The degree of hydration was calculated using the average diameter measured at three positions along the length of the fibre using digital calipers. The average diameter of the wet fibre was compared against the diameter of the dry fibre as a measure of the degree of hydration as represented in Equation 5.1.

$$Degree\ of\ hydration = \frac{Diameter\ of\ wet\ fiber - Diameter\ of\ dry\ fiber}{Diameter\ of\ the\ dry\ fiber} \times 100 \qquad \textit{Equation 5.1}$$

5.3.8. Characterisation of vibrational transitions

Chemical interactions between polymer, crosslinking salt, plasticiser and model drugs were investigated using Fourier Transform Infrared (FTIR) Spectroscopy using a 100FT-IR Spectrometer (PerkinElmer Inc., Waltham, Massachusetts, USA). The reference spectrum used was air. Samples tested included drug-free and drug-loaded polymeric fibres. Changes in vibrational absorption occur as new bonds are formed altering the infra red spectra which were qualitatively compared against one another. Formulation components and model drugs were run individually such that the sample spectra were compared to standard spectra.

5.3.9. Static lattice atomistic stimulations relating to Young's modulus

The effect of varying the plasticiser and crosslinking ion concentration on Young's modulus and ultimate strain of the fibres and the performance of the ciprofloxacin- and/or diclofenac-loaded optimised fibre was further elucidated and conceptualised using Molecular Mechanics Energy Relationships (MMER) by exploring the spatial disposition of energy minimised molecular structures. All modelling procedures and calculations, including energy minimisations in molecular mechanics, were performed using the HyperChem^{TM} 8.0.8 Molecular Modeling System (Hypercube Inc., Gainesville, Florida, USA) and ChemBio3D Ultra 11.0 (CambridgeSoft Corporation, Cambridge, UK) on an HP Pavilion dv5 Pentium Dual CPU T3200 workstation. The structures of alginate – Alginate$_{10}$ (Alg$_{10}$: ten oligosaccharide units for tensile analysis of preoptimised batches) and Alginate$_4$ (Alg$_4$: four oligosaccharide units for drug loaded optimised batch) were generated (1,4-linked β-D-mannuronate:α-L-guluronate, 1:1) using sugar builder module on HyperChem 8.0.8. Structures of Glycerol (Gly), Ciprofloxacin (Cpr) and Diclofenac (Dcl) were built up with natural bond angles as defined in the Hyperchem software. The models were initially energy-minimised using MM+ force field and the resulting structures were again energy-minimised using the Amber 3 (Assisted Model Building and Energy Refinements) force field. The conformer having the lowest energy was used to create the polymer-plasticiser, polymer-crosslinker and polymer-drug complexes. A complex of one molecule with another was assembled by disposing the molecules in a parallel way, and the same procedure of energy-minimisation was repeated to generate the final models: Alg-Gly, Alg-Ba^{2+}, Alg-Gly-Ba^{2+}, Cpr-Dcl, Alg-Cpr, Alg-Dcl and Alg-Cpr-Dcl. Full geometry optimisation was carried out in vacuum employing the Polak–Ribiere conjugate gradient algorithm until an RMS gradient of 0.001kcal/mol was reached. Force field options in the AMBER (with all hydrogen atoms explicitly included) and MM+ (extended to incorporate non-bonded cut-offs and restraints) methods were the HyperChem 8.0.8 defaults (Kumar *et al.*, 2011). A molecular mechanics conformational searching procedure was employed to acquire the data employed in the statistical mechanics analysis, and to obtain differential binding energies of a Polak–Ribiere algorithm and to potentially permit application to polymer composite assemblies. MM+ is a HyperChem modification and extension of Norman Allinger's Molecular Mechanics program MM2 (Warhurst *et al.*, 2003), whereas AMBER, is a package of computer programs for applying molecular mechanics, normal mode analysis, molecular dynamics and free energy calculations to simulate the structural and energetic properties of molecules (Pearlman *et al.*, 1995).

5.4. Results and Discussion

5.4.1. Morphological and surface structure analysis

Analysis of SEM images revealed the interaction between the model drugs and the polymeric fibre as depicted in Figure 5.1. A uniform cylindrical filamentous structure is evident in the drug-free formulation (Figure 5.1d). Images of fibres loaded with ciprofloxacin and diclofenac sodium (Figure 5.1a and b respectively) display a similar structure to the drug-free fibre. However, when these two drugs are simultaneously loaded into the fibre the structure is disrupted as represented in Figure 5.1c. The surface of ciprofloxacin and diclofenac sodium-loaded fibre appears uneven and less aligned in structure compared to the other formulations.

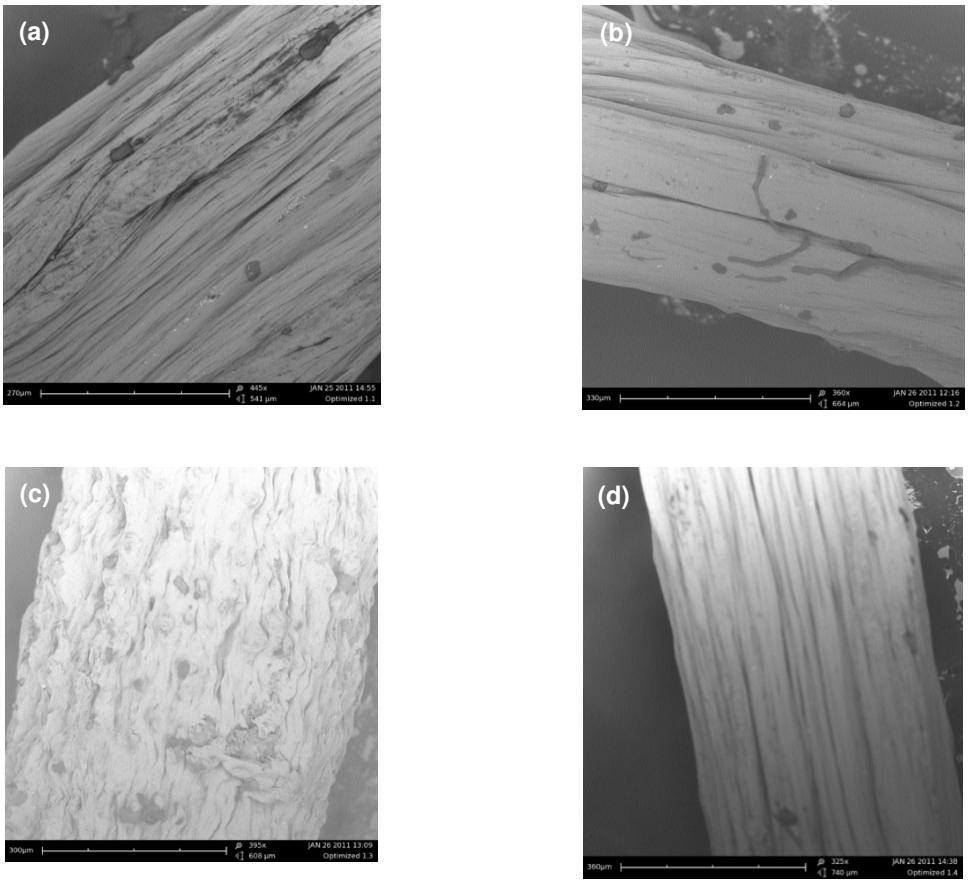


Figure 5.1: SEM images (a) ciprofloxacin-loaded fibre, (b) diclofenac sodium-loaded fibre, (c) ciprofloxacin/diclofenac-loaded fibre, (d) drug-free fibre

5.4.2. UPLC method development for the co-detection of ciprofloxacin and diclofenac sodium

The UPLC builds on the technology of high performance liquid chromatography (HPLC) analysing compounds with increased speed, sensitivity and resolution while drastically reducing run times and has significantly improved processes within drug delivery (Swartz, 2005). Inclusion of ciprofloxacin and diclofenac within the same fibre necessitated the development of a chromatographic method for the co-detection of these two drugs from dissolution samples. The reverse phase chromatographic method analyses the chemical structures affinity for the non-polar stationary phase or the more polar mobile phase. Using a gradient method, the composition of the mobile phase was adjusted to ensure that both drugs produced narrow peaks without visible tailing and that these peaks were easily resolved from one another.

The three dimensional analysis of the PDA spectrum from λ=220-400nm identified that ciprofloxacin was eluted before diclofenac sodium with retention times of 0.8 minutes and 1.7 minutes respectively, depicted in Figure 5.2. Adequate separation of the two peaks as well as the maximum peak for both drugs was between 270-280nm, therefore the channel was set at λ=278nm for analysis.

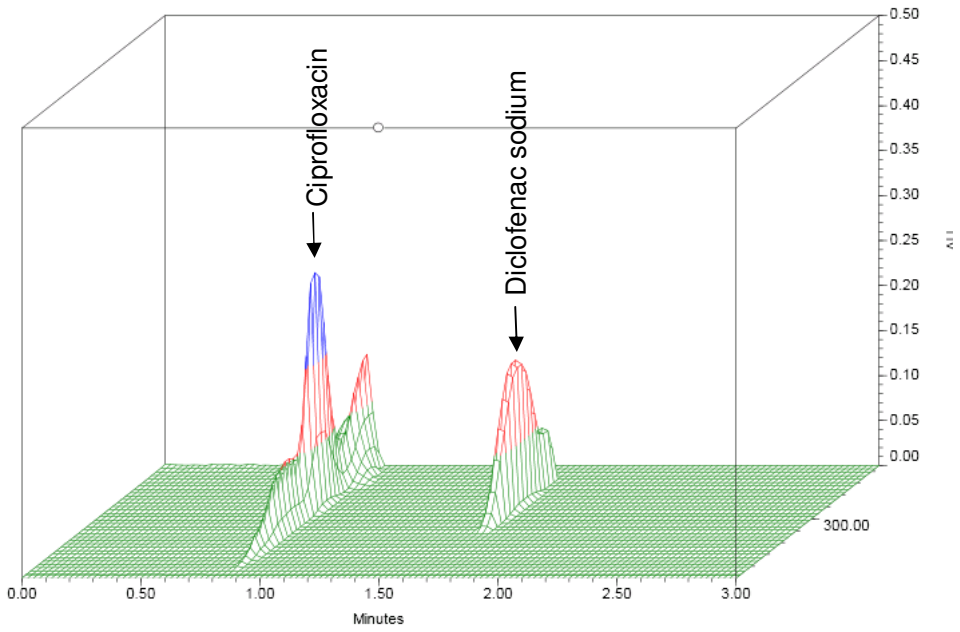


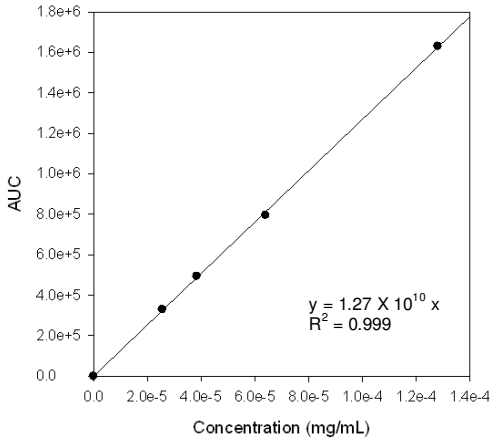
Figure 5.2: PDA spectrum for the detection of ciprofloxacin and diclofenac sodium at approximately 0.8 and 1.7 minutes respectively

5.4.3. Drug entrapment efficiency of fibres

The USP (1995) states that both peak height and area can be related to analyte concentration, however, peak areas have been considered a more accurate method for quantification. Resolution between peaks was sufficient; consequently the peak areas were used to quantify the concentration of ciprofloxacin and diclofenac sodium in both standards and samples.

Two calibrations curves for ciprofloxacin and diclofenac sodium, shown in Figure 5.3a and b respectively, were used to determine the drug entrapment within the optimised fibre, plotting the area under the curve (AUC) versus the concentration of either ciprofloxacin or diclofenac sodium. The incremental increases in absorbance and thus area, is noted in Figure 5.4, representing an increase in concentration of drug with each standard sample injected.

(a) Ciprofloxacin



(b) Diclofenac sodium

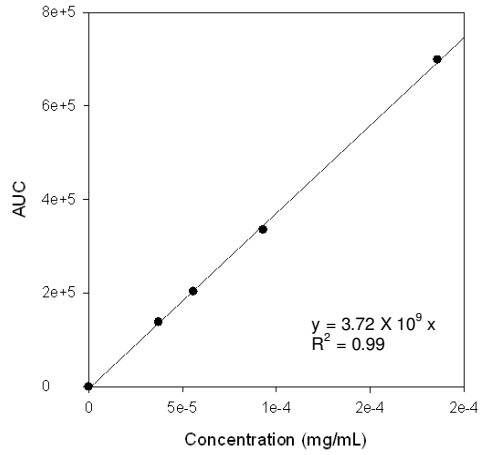


Figure 5.3: Calibration curve in PBS pH 8 for (a) ciprofloxacin and (b) diclofenac sodium

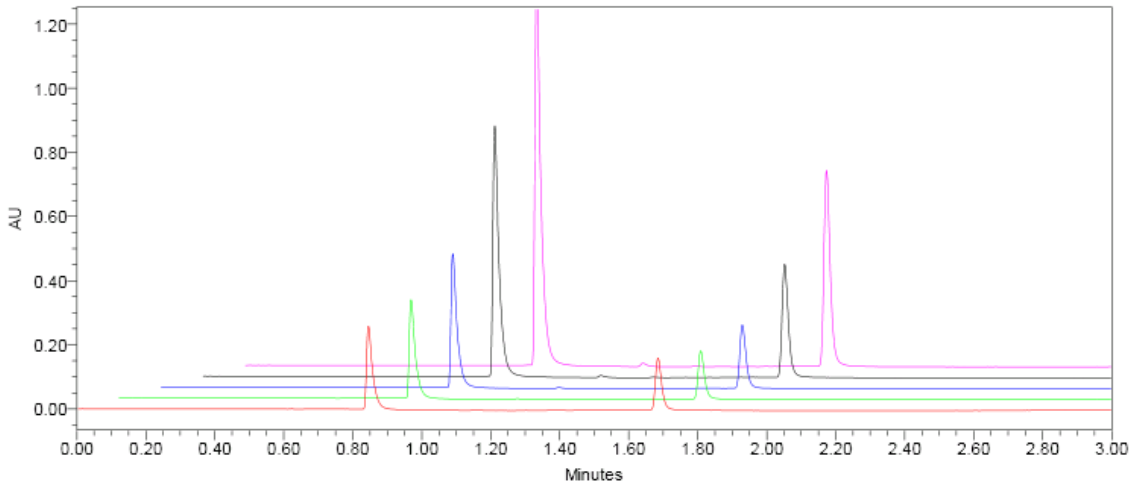


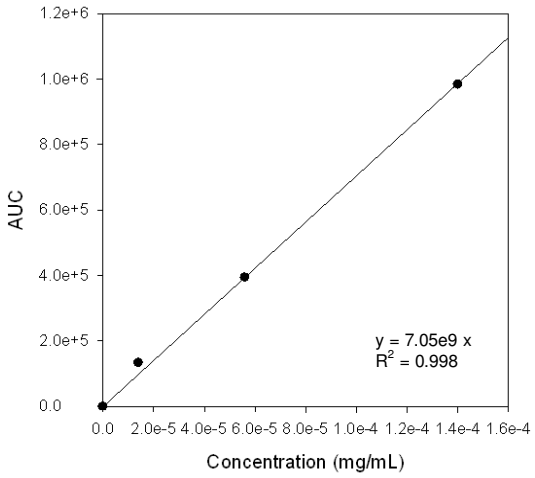
Figure 5.4: Overlaid chromatograms representing the serial dilutions where the area under the curve at 0.8 minutes and 1.7 minutes was used in determining the calibration curves for ciprofloxacin and diclofenac sodium respectively

It was uncertain if both drugs would remain entrapped within the fibre when combined simultaneously in the same formulation. Drug entrapment samples that were taken from drug-loaded fibres dissolved in PBS pH 8 confirmed that both ciprofloxacin and diclofenac were successfully retained within the formulation at 79.43% and 95.78% correspondingly.

5.4.4. *In vitro* drug release behaviour

For accurate analysis the analyte needs to be soluble in the mobile phases, hence for analysis of dissolution samples, standard samples of ciprofloxacin and diclofenac sodium were dissolved in water and acetonitrile at a ratio of 1:1, which was completely miscible with the mobile phases over the three minute run time. The AUC was calculated and plotted against drug concentration giving calibration curves for ciprofloxacin and diclofenac sodium in Figure 5.5a and b respectively.

(a) Ciprofloxacin



(b) Diclofenac sodium

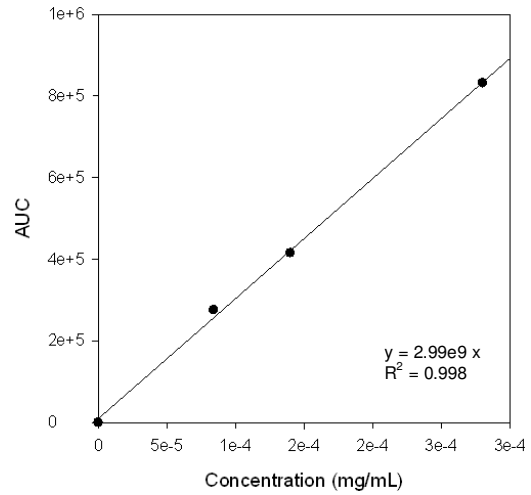


Figure 5.5: Calibration curves in acetonitrile:water (50:50) for assessment of (a) ciprofloxacin and (b) diclofenac sodium release

Drug release results of the optimised formulation revealed critical information regarding the successful formulation of the PFD. Drug interactions between ciprofloxacin and diclofenac affecting release kinetics as well as capacity of the fibre to retain these two drugs in sufficient concentrations were two major concerns of simultaneous drug entrapment. The fractional drug release over 10 days (Figure 5.6) confirmed that ciprofloxacin was consistently released over 10 days at both pHs tested. Diclofenac sodium was poorly released in PBS pH 4, but was steadily released in PBS pH 6.8 over the same time period. These results are visually represented in the stacked chromatograms representing drug release at each time point in PBS pH 4 and pH 6.8 correspondingly in Figures 5.7 and 5.8. The peak at approximately 1.7 minutes, characterising diclofenac release, is absent in Figure 5.7, however, it is present in the chromatograms at each time interval from day 1 to day 10 in Figure 5.8. Comparatively peaks at approximately 0.8 minutes represent ciprofloxacin released at pH 4 and pH 6.8 and is present at each time interval tested.

Drug release kinetics has been reported to depend on drug solubility, pH of dissolution medium and changes in the crystalline structure of the matrix upon hydration (Hodsdon *et al.*, 1995). Furthermore, Bajpai and Sharma (2004) stated that alginate in an acidic environment undergoes hydrolysis to for alginic acid leading to reduced mechanical strength and a degree of crosslinking. These explanations could account for the release of ciprofloxacin and diclofenac in PBS pH 4 and pH 6.8 with or without swelling of the polymeric matrix. Ciprofloxacin, a weak basic drug, was released to a greater extent in PBS pH 4 as it is more soluble in acidic media compared to PBS pH 6.8 where it is less soluble. However, it is suggested that the swelling and relaxation of the fibre accounted for the ciprofloxacin's release in PBS pH 6.8. In contrast diclofenac sodium, a weak acidic drug, released 80% of entrapped the entrapped drug in PBS pH 6.8. Exceptionally low levels of diclofenac were released in PBS pH 4, which can be hypothesised as being due to the drugs poor solubility in acidic media in conjunction with reduced swelling and relaxation of the PFD in PBS pH 4.

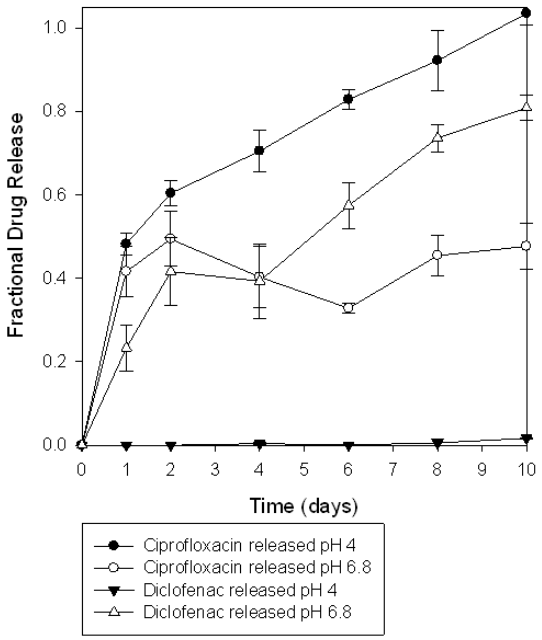


Figure 5.6: Simultaneous fractional drug release of ciprofloxacin and diclofenac sodium from the optimised fibre formulation (N=3)

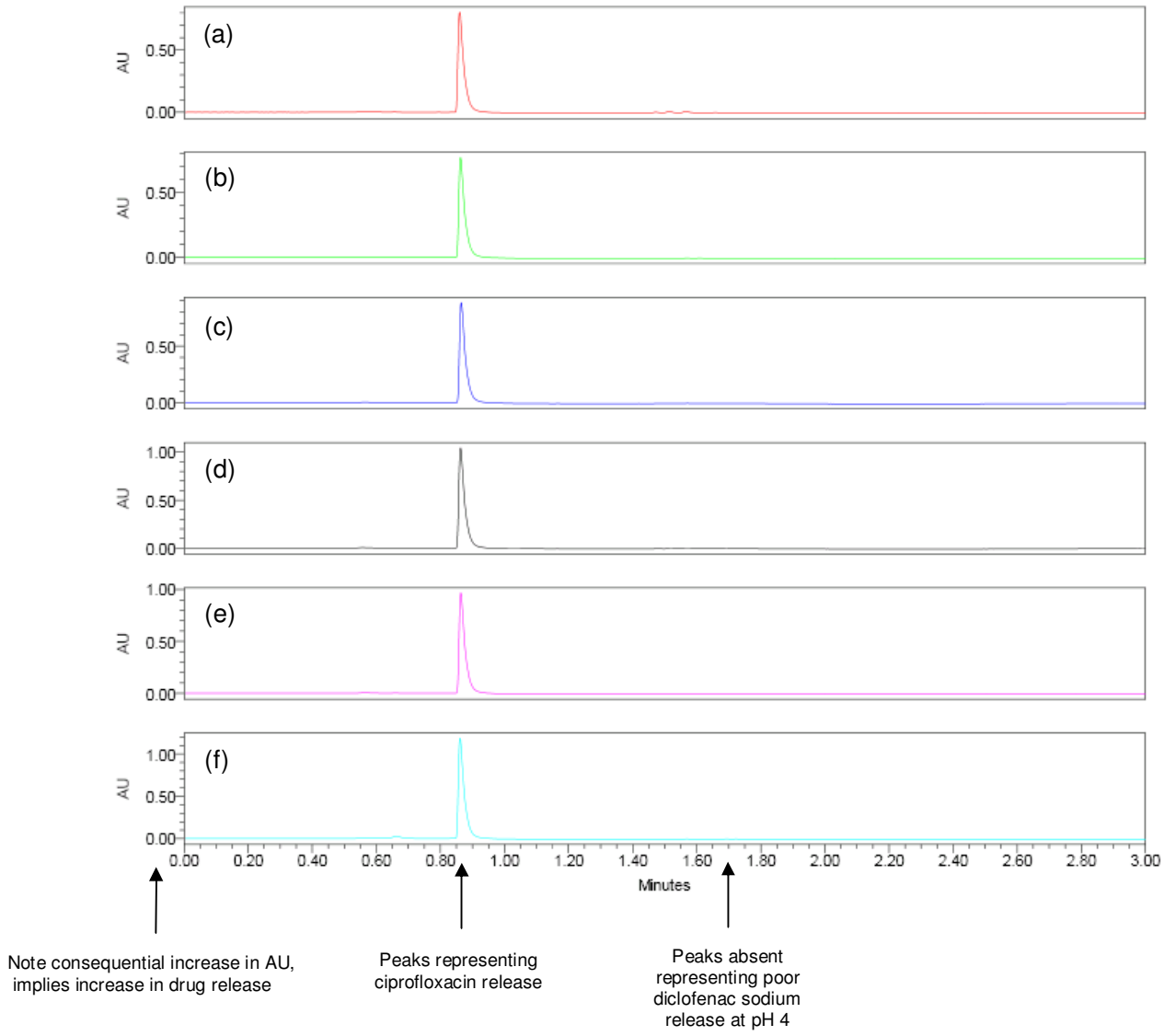


Figure 5.7: Stacked chromatograms of dissolution samples in PBS pH 4 on day (a) 1, (b) 2, (c) 4, (d) 6, (e) 8 and (f) 10 at λ=278nm

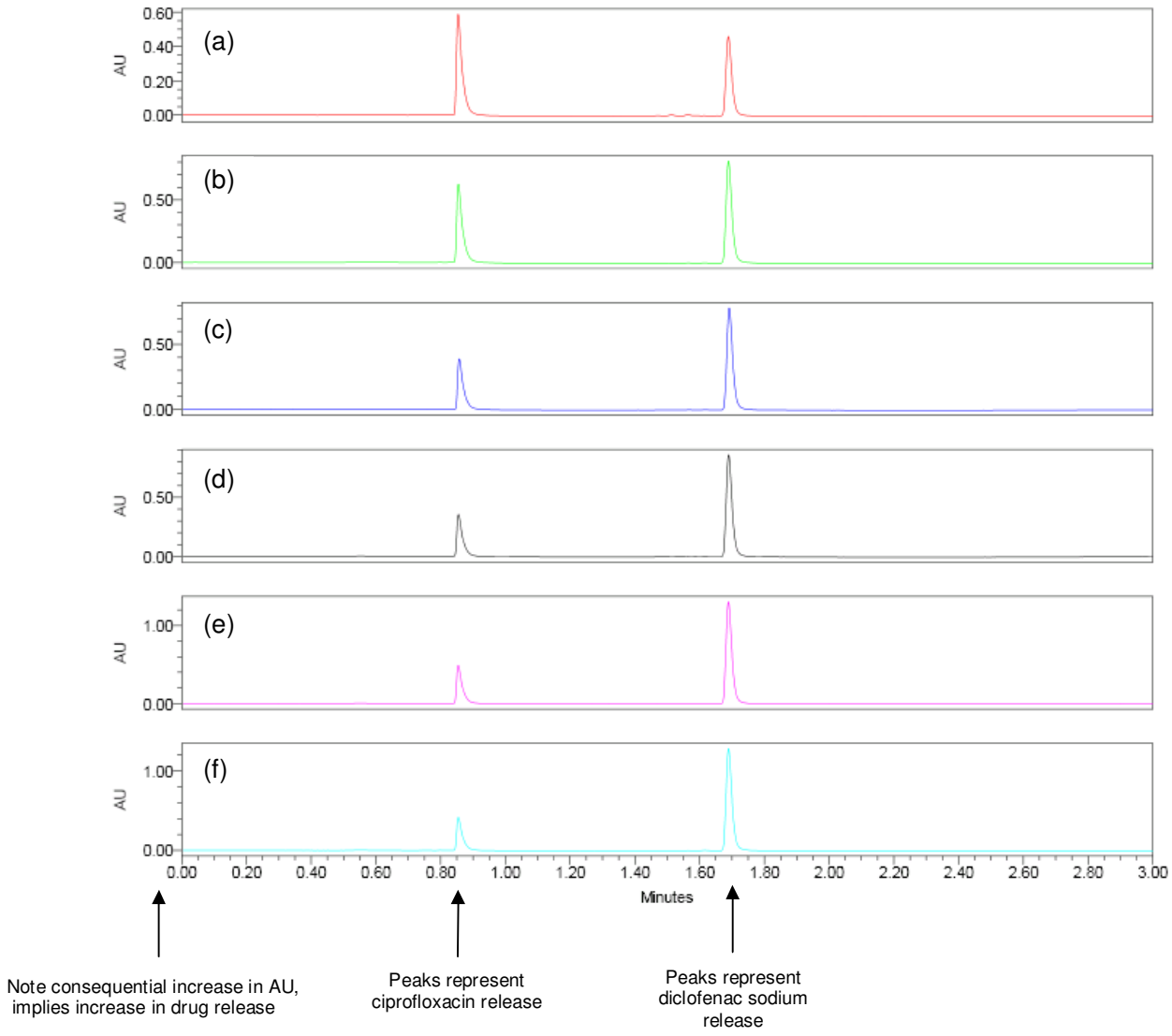


Figure 5.7: Stacked chromatograms for dissolution samples in PBS pH 6.8 on day (a) 1, (b) 2, (c) 4, (d) 6, (e) 8 and (f) 10 at λ=278nm

The MDT for ciprofloxacin and diclofenac sodium in PBS pH 4 and 6.8 is summarised in Table 5.3. In comparison to the MDT for optimised fibres with either ciprofloxacin or diclofenac sodium loaded within the fibre reported at the end of Chapter 4, a notable reduction in MDT $_{10\ days}$ for both drugs at both pH's was noted. A reduction in MDT validated the proposition that the matrix retards the release of both the drugs. This may too be attributed to the more sensitive method of quantifying drug release using UPLC compared to a UV.

Table 5.3: MDT$_{10\ days}$ for ciprofloxacin and diclofenac release in PBS pH 4 and pH 6.8

Days	*MDT $_{10\ days\ ciprofloxacin}$*		*MDT $_{10\ days\ diclofenac\ sodium}$*	
	pH 4	*pH 6.8*	*pH 4*	*pH 6.8*
0	0	0	0	0
1	0.24	0.21	0	0.11
2	0.91	0.74	0	0.62
4	2.11	1.21	0.012	1.18
6	4.14	1.64	0.002	2.87
8	6.45	3.18	0.04	5.15
10	9.31	4.29	0.15	7.28

5.4.5. Textural analysis

Jejurikar *et al.* (2011) examined tensile curves of alginate hydrogels and found a two-stage deformation profile. The steep first part of the curve represents an elastic response with some stretching until a yield value is reached after which plastic deformation occurs where disruption in crosslinking bonds and chain sliding occurs. Tensile analysis of drug-free and drug-loaded optimised fibres revealed profiles for further analysis, as depicted in Figure 5.9.

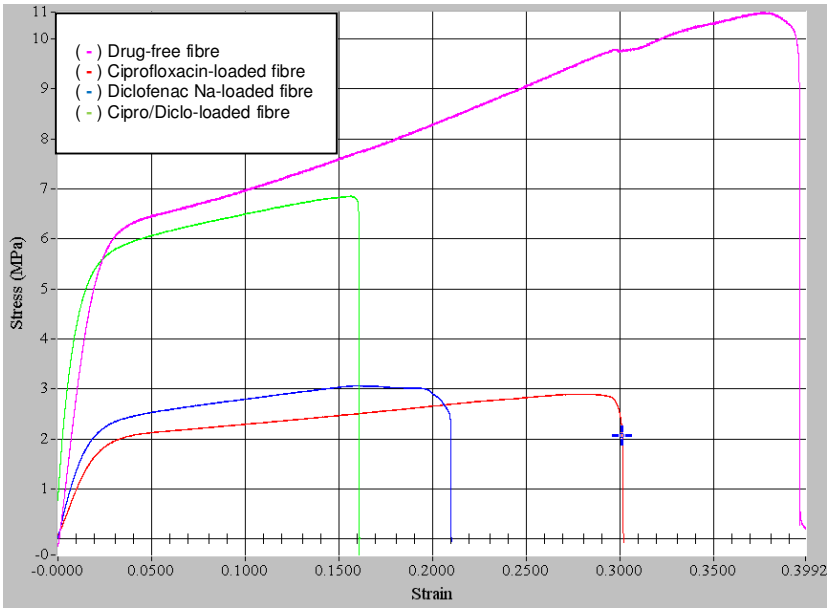


Figure 5.9: Stress versus strain profiles for drug-free and drug-loaded fibres

Two important tensile properties, strength and flexibility, are vital in the analysis of fibres for placement within the periodontal pocket. Firstly, fibres need to be tough enough enabling handling and processing without breakage. The tensile strength of the fibres were attributed to both the type and concentration of polymer and crosslinking salt incorporated. Drury *et al.* (2004) investigated the tensile properties of alginate based hydrogels and deduced that higher G monomers enhance the tensile properties within the polymeric backbone. The high tensile strength may also be be attributed to barium's large ionic radii, being responsible for the stronger crosslinked hydrogel formed compared to other divalent cations such as calcium (Jejurikar *et al.,* 2011). Secondly, fibres require a particular degree of flexibility for manipulation during placement within the periodontal pocket. Glycerol was included in the formulation as a plasticiser for this reason. Avella *et al.* (2007) researched the interaction between glycerol and alginate and deduced that the flexibility of the matrix attributed to the structure of the polysaccharide used, where higher molecular weight and higher amounts of G monomers present leads to entanglement of glycerol within the polymeric structure. Furthermore, their results revealed a significant drop in Young's modulus values and stress at breakage, while an increase in the strain at breakage values, with decreasing sodium alginate and increasing glycerol concentrations.

Following the experimental design, polymer, crosslinking salt and plasticiser concentrations were optimised and were held constant while comparing the interaction of the two drugs, ciprofloxacin and diclofenac sodium with fibre components. Drug-free fibres were compared to diclofenac-loaded, ciprofloxacin-loaded and ciprofloxacin/diclofenac-loaded fibres. A visual comparison of these formulations is seen in the stress vs. strain plots with the four curves superimposed on each other and in the force vs. displacement profiles, in Figure 5.9 and 5.10 respectively. Drug-free fibres withstood the highest stress and strain before fracturing compared to drug-loaded fibres as reviewed in Table 5.4. Incorporation of either diclofenac sodium or ciprofloxacin within the fibres reduces the strength and flexibility of the formulation. Surprisingly as seen in the typical force versus displacement profiles (Figure 5.10b) when the two drugs were combined, the resultant drug-loaded fibre fractures at a higher stress value (1205mN) while still stretching 3.457 mm in length before fracturing. These observations are consistent with the higher Young's modulus values (326.34±37.91MPa) calculated for the ciprofloxacin/diclofenac sodium loaded fibers and lower ultimate strain values (0.15±0.05). Both diclofenac-loaded and ciprofloxacin-fibres fractured at considerably lower forces with Young's modulus values plummeting to 78.02 and 132.87MPa respectively.

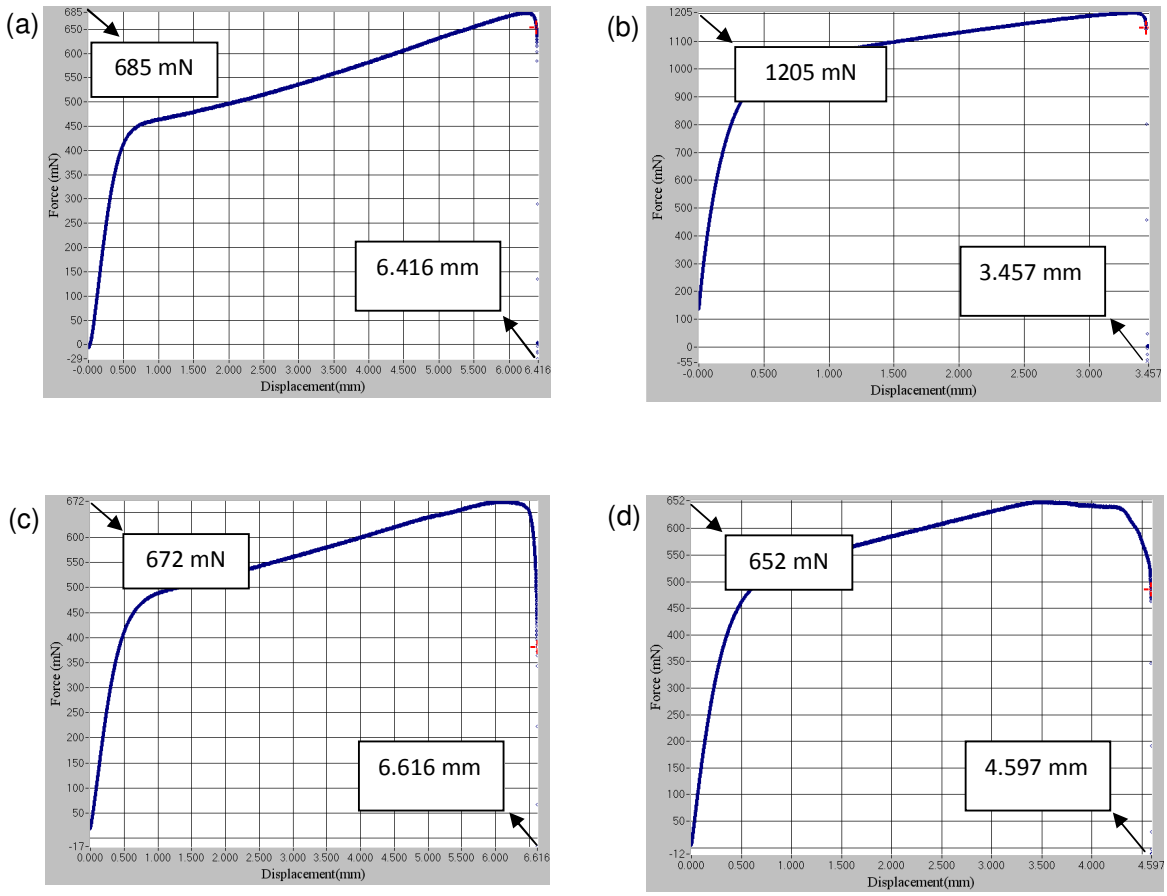


Figure 5.10: Typical profiles detailing the relationship between force (mN) and displacement (mm) for (a) drug-free fibres, (b) ciprofloxacin/diclofenac-loaded fibres, (c) ciprofloxacin-loaded fibres and (d) diclofenac-loaded fibres

Table 5.4: Tensile analysis results comparing drug-free fibres to drug-loaded fibres (N=5)

	Drug-free fibres	*Ciprofloxacin-loaded fibres*	*Diclofenac-loaded fibres*	*Ciprofloxacin/diclofenac -loaded fibres*
Young's modulus (MPa)	314.04±18.93	78.02±9.65	132.87±37.23	326.34±37.91
Yield stress (MPa)	5.80±0.40	1.51±0.23	2.02±0.51	4.25±0.84
Ultimate strength (MPa)	10.05±2.09	2.97±0.30	3.50±0.60	6.49±1.68
Ultimate strain	0.29±0.12	0.83±1.11	0.26±0.10	0.15±0.05
Toughness (Jcm^{-3})	2.39±1.25	0.74±0.07	0.71±0.18	0.93±0.49

5.4.6. Swelling and erosion analysis

Alginate interacts with divalent cations resulting in gelation or precipitation while a soluble salt complex is formed when monovalent cations interact with the polymer (Tønnesen and Karlsen, 2002). Crosslinker density, alginate concentration and alginate chemical composition have been identified as factors affecting swelling behaviour of crosslinked alginate hydrogels (Kuo and Ma, 2007).

Alginate based polymeric fibres initially swelled in PBS pH 6.8 up to 300% in the first day, after which the matrix eroded steadily over the next few days. In comparison the fibre does not swell in PBS pH 4 but slowly erodes. Swelling of crosslinked alginate matrices in PBS is explained as a result of the divalent crosslinked ions being replaced by sodium ions present in solution, resulting in repulsion of the $-COO^-$ groups, chain relaxation and swelling (Bajpai and Sharma, 2004).

The degree of hydration varied considerably in PBS at pH 6.8 between the drug loaded and drug-free samples as depicted in Figure 5.11. Ciprofloxacin/diclofenac-loaded fibres swelled to almost twice as much as drug-free fibres. This may perhaps be ascribed to the degree of crosslinking and porosity of the matrix interacting with the sodium ions present in solution.

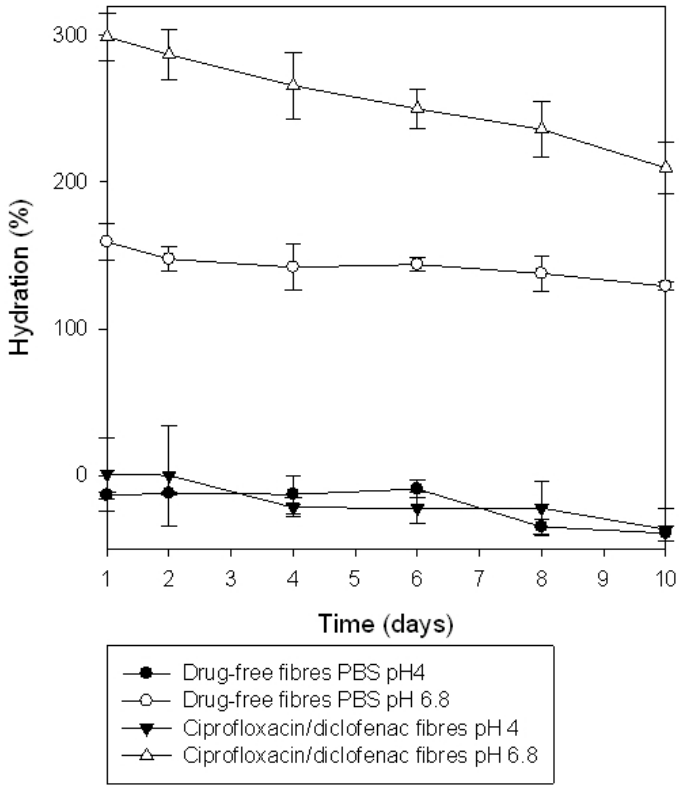


Figure 5.11: The hydration behaviour drug-free and ciprofloxacin/diclofenac-loaded PFD over 10 days in PBS pH4 and 6.8 (N=3)

5.4.7. Vibrational transition analysis

Functional groups within molecules experience vibrational excitation and absorb infrared radiation corresponding to the vibrational energies. The absorption spectra are a blue print of the molecular structure and can therefore be used in the analysis of an unknown spectrum to reference spectrum (Coates, 2000). The unknown spectra analysed were drug-free and drug-loaded co-blended crosslinked alginate based fibres, illustrated in Figure 5.12. These spectra were compared against spectra of the individual components employed in the formulation of the fibres namely alginate, barium chloride 2-hydrate and glycerol as depicted in Figure 5.13.

The analysis of the FTIR spectrum revealed broad peaks present in all fibres ranging from 3260.70-3273.79cm^{-1}, which characterises the presence of O-H groups. C-H stretching (2850-3000cm^{-1}) was present in the glycerol spectra with two peaks visible at 2932.32cm^{-1} and 2879.69cm^{-1}. Similar peaks were present in all fibre formulations shifting towards slightly higher wavelengths. Two unknown peaks at 1635.68cm^{-1} and 1599.62cm^{-1} were present in the barium chloride spectra. The crosslinking between alginate and barium chloride was observed by Lawrie *et al.* (2007) identifying the antisymmetric carbonyl vibration (1596cm^{-1}) been most sensitive to addition of barium chloride ions. Similarly, in Figure 5.13, the peak present in the sodium alginate spectra at 1594.28cm^{-1} was absent or had shifted in the spectra in Figure 5.12. A comparable peak at approximately 1640cm^{-1} was observed in all four fibre formulations which may reflect the unknown peak from the barium chloride spectra or a shift in the carbonyl vibration present in sodium alginate. Furthermore, a shift in the bands to higher or lower energies may have occurred due to sodium alginate crosslinking with divalent ions which is dependent on the M/G monomer ratio (Lawrie *et al.*, 2007). Shifts in peaks at 1405.28cm^{-1} and 1024.82cm^{-1} in the sodium alginate spectrum to 1413.17-1415.80cm^{-1} and 1036.27-1037.87cm^{-1} respectively, further reflected in the interaction between barium chloride and alginate. The shift in peak at 1030cm^{-1} was noted by Sakugawa *et al.* (2004) reflecting a change in M monomer concentration in the presence of calcium ions, confirming the shifts in peaks evident between 1024.82cm^{-1} and approximately 1037cm^{-1} as an interaction between the polymer and crosslinking salt.

Incorporation of ciprofloxacin and diclofenac, individually or in combination within the fibre did not result in any significant changes in the spectra when compared to the drug-free fibres. If no chemical interactions between the polymer and drug are evident, the release will depend on the hydrophobicity of the drug diffusing through the gel pores (Augst *et al.*, 2006).

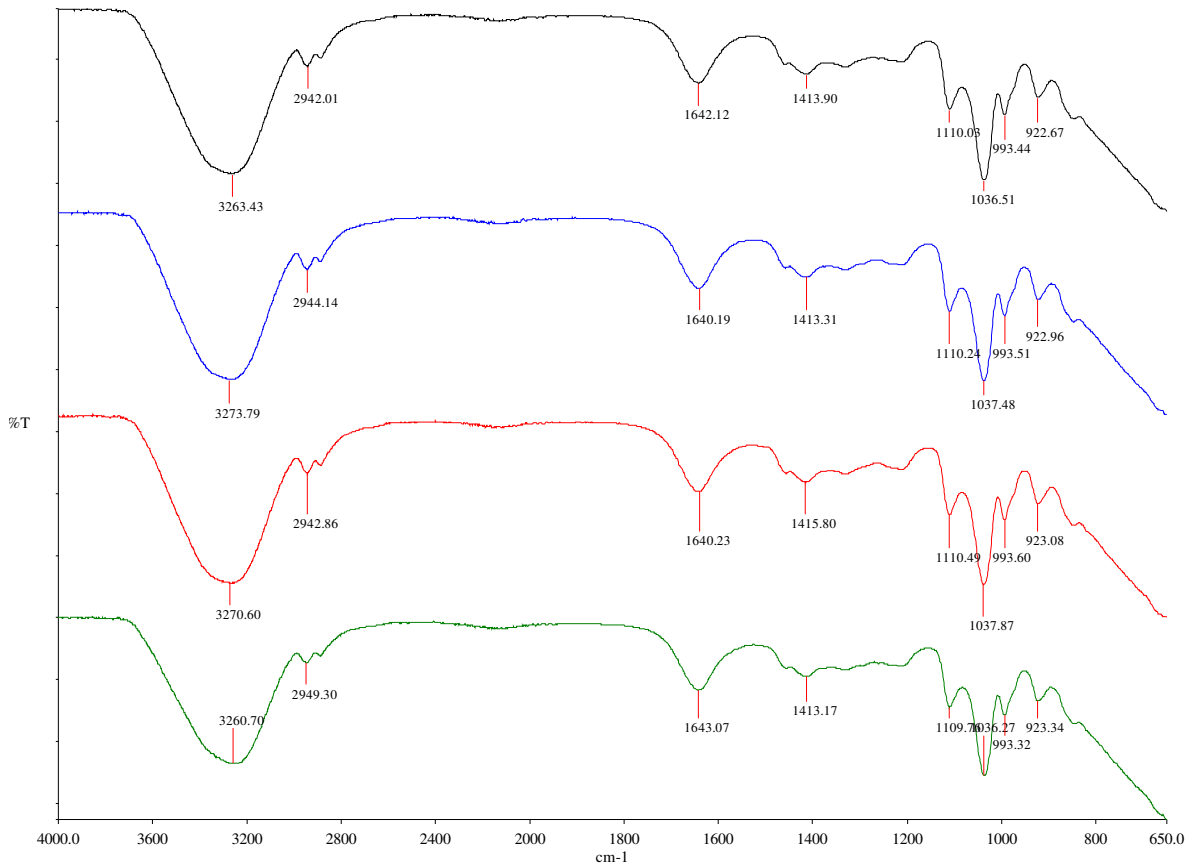


Figure 5.12: FTIR spectrum representing (–) drug-free fibres, (–) ciprofloxacin-loaded fibres, (–) diclofenac sodium-loaded fibres and (–) ciprofloxacin/diclofenac-loaded fibres

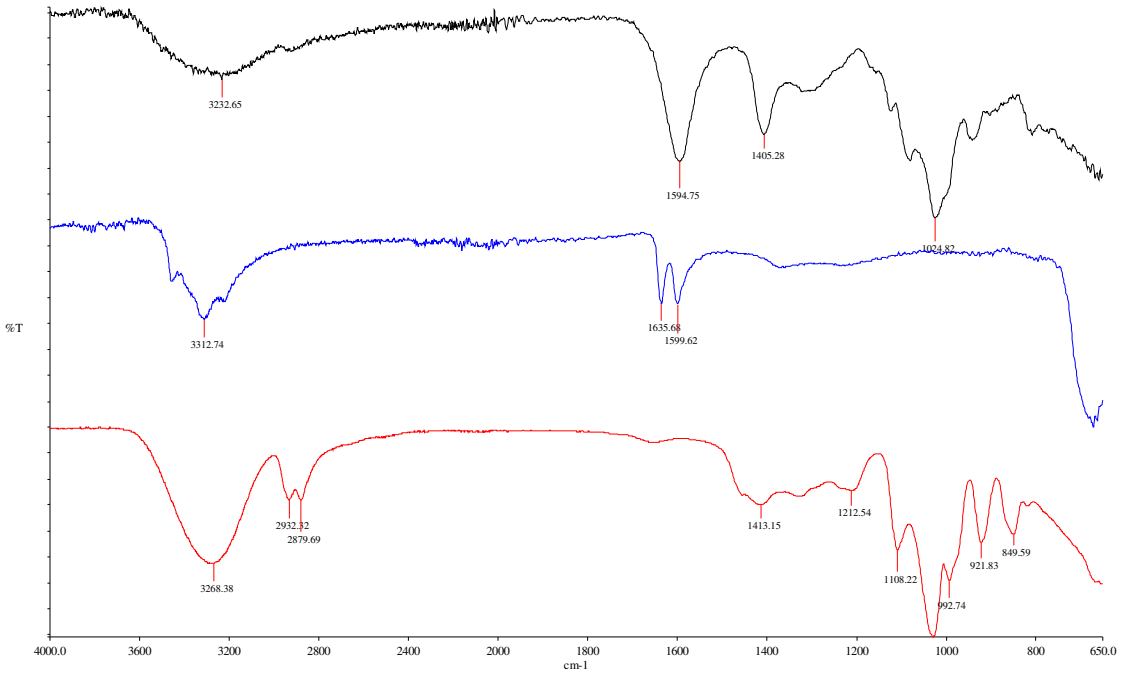


Figure 5.13: FTIR spectrum of (–) alginate, (–) barium chloride 2-hydrate and (–) glycerol

5.4.8. Static lattice atomistic simulations

Molecular mechanics energy relationship (MMER), a method for analytico-methematical representation of potential energy surfaces, was used to provide information about the contributions of valence terms, noncovalent Coulombic terms, and noncovalent van der Waals interactions in mechano-tensile properties and drug incorporation effect. The MMER model for potential energy factor in various molecular complexes can be written as:

$$E_{molecule/complex} = V_{\Sigma} = V_b + V_\theta + V_\varphi + V_{ij} + V_{hb} + V_{el} \quad \textit{Equation 5.2}$$

where, V_{Σ} is related to total steric energy for an optimised structure, V_b corresponds to bond stretching contributions (reference values were assigned to all of a structure's bond lengths), V_θ denotes bond angle contributions (reference values were assigned to all of a structure's bond angles), V_φ represents torsional contribution arising from deviations from optimum dihedral angles, V_{ij} incorporates van der Waals interactions due to non-bonded interatomic distances, V_{hb} symbolises hydrogen-bond energy function and V_{el} stands for electrostatic energy (Choonara *et al.*, in press)

In the present molecular modeling study, the global energy relationships for the various complexes derived after assisted model building and energy refinements were as follows:

$$E_{ALG\text{-}10} = 74.426V_{\Sigma} = 5.204V_b + 32.485V_\theta + 34.168V_\varphi + 30.709V_{ij} - 28.142V_e \quad \textit{Equation 5.3}$$

$$E_{GLY\text{-}5} = 14.315V_{\Sigma} = 0.230V_b + 1.345V_\theta + 10.615V_\varphi + 2.185V_{ij} - 0.065V_{hb} \quad \textit{Equation 5.4}$$

$$E_{ALG\text{-}GLY5} = 22.558V_{\Sigma} = 5.698V_b + 32.688V_\theta + 47.908V_\varphi + 17.697V_{ij} - 1.454V_{hb} - 79.979Vel \quad \textit{Equation 5.5}$$

$$E_{ALG\text{-}Ba^{2+}10} = 38.839V_{\Sigma} = 5.662V_b + 31.068V_\theta + 40.489V_\varphi + 36.363V_{ij} - 74.744V_{el} \quad \textit{Equation 5.6}$$

$$E_{ALG\text{-}GLY5\text{-}Ba^{2+}10} = 14.917V_{\Sigma} = 5.641V_b + 27.792V_\theta + 38.072V_\varphi + 24.932V_{ij} - 1.456V_{hb} - 80.065V_e \quad \textit{Equation 5.7}$$

$$E_{ALG\text{-}4} = -12.974V_{\Sigma} = 2.562V_b + 22.378V_\theta + 37.927V_\varphi + 16.696V_{ij} - 4.584V_{hb} - 87.954V_e \quad \textit{Equation 5.8}$$

$$E_{CPR} = 118.194V_{\Sigma} = 0.989V_b + 99.998V_\theta + 9.149V_\varphi + 8.063V_{ij} - 0.006V_{hb} \quad \textit{Equation 5.9}$$

$$E_{DCL} = 7.483V_{\Sigma} = 0.668V_b + 3.845V_\theta + 0.814V_\varphi + 2.434V_{ij} - 0.28V_h \quad \textit{Equation 5.10}$$

$$E_{ALG\text{-}CPR} = 84.568V_{\Sigma} = 3.552V_b + 120.693V_\theta + 47.076V_\varphi + 12.08V_{ij} - 4.375V_{hb} - 94.559V_{el} \quad \textit{Equation 5.11}$$

$$E_{ALG\text{-}DCL} = -17.938V_{\Sigma} = 3.107V_b + 27.608V_\theta + 37.187V_\varphi + 5.907V_{ij} - 3.903V_{hb} - 87.845V_{el} \quad \textit{Equation 5.12}$$

$$E_{ALG\text{-}CPR/DCL} = 422.162V_{\Sigma} = 220.235V_b + 81.482V_\theta + 23.052V_\varphi + 136.476V_{ij} - 1.629V_{hb} - 37.454V_{el.} \quad \textit{Equation 5.13}$$

5.4.8.1. Formulation and crosslinking of plasticised-alginate fibres

The present work focused on the fabrication of a polysaccharide (alginate) based macro-fibrous system integrating a unique combination of plasticiser (glycerol) and crosslinker (divalent barium ions). The energy changes brought about by crosslinking of plasticised alginate system and the resulting geometrical minimisations are depicted in Figure 5.14. In accordance with the sequence of steps in Chapter 3 Section 3.3.1, alginate solution was first plasticised with addition of glycerol and thereafter crosslinked in barium chloride solution in the form of fibres. Although the tensile analysis was conducted on crosslinked-plasticised fibre, the effect of addition of the individual component to the alginate during the initial stage of static simulations is elucidated upon.

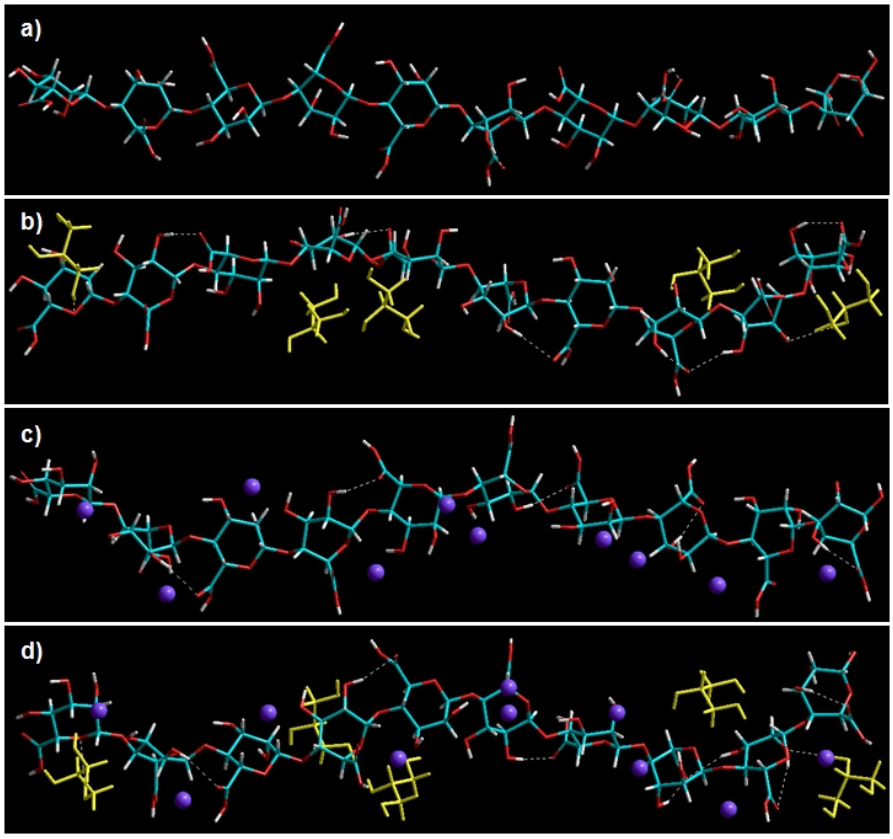


Figure 5.14: Visualisation of energy minimised geometrical preferences of a) alginate (10 monosaccharide units); b) alginate-glycerol; c) alginate-Ba^{2+}; and d) alginate-glycerol-Ba^{2+} showcasing the intra- and inter- molecular interactions in crosslinked-plasticised-alginate fibres after molecular simulations in vacuum. Glycerol molecules are rendered as tubes in yellow and barium ions are rendered spherically in violet. Colour codes for elements: C (cyan), O (red), N (blue), and H (white)

The incorporation of glycerol to alginate conceded the standards of polymer-plasticiser combination, both kinetically and thermodynamically. Kinetically, glycerol dissolved rapidly into the alginate solution and in the thermodynamic sense, the free energy of mixing or the change in total steric energy of complex was negative (-66.183kcal/mol; Eqns. 2-4; Table I), thereby forming a highly stable preferential molecular composite with intra- and inter-molecular H-bonds ranging from 2.2052A° to 3.0464A° (avg. 2.4647A°). Furthermore, glycerol displayed strong interactions with the polysaccharide as displayed in Figure 5.14.

Alginates are known to exhibit interactions with various divalent metal ions, such as calcium and barium, where they display concentration-dependent intra- and inter-molecular crosslinking and complexation of the polymer matrix (da Silva *et al.*, 2009). It is evident from Table 5.5, Alg-Ca^{2+} was energetically stabilised by 35.587kcal/mol (Eqns. 2 and 5) as compared to alginate because of strong electrostatic interactions (ΔE = -46.602kcal/mol) along with high torsional energy (ΔE = 6.321kcal/mol) leading to the formation of a strained network structure - with smaller intramolecular H-bonds ranging from 2.208A° to 3.3595A° (avg. 2.2996A°) - due to calcium crosslinking thereby limiting the complete interaction as observed with the non-crosslinked structure (Figure 5.14). These results are in line with the earlier reported studies where the polyguluronates (alginate) displayed better strength and stereospecificity in binding to calcium through “Egg-box model” (Braccini and Pérez, 2001).

The crosslinked-plasticised polymeric matrix, Alg-Gly-Ba^{2+}, formed by ionic crosslinking and subsequent gelation of already plasticised alginate displayed a well organised structure with formation of several intra- and inter-molecular H-bonds (although at different positions as compared to bimolecular structures) having bond length ranging from 2.2051A° to 3.1906A° with (avg. 2.5486A°). The trimolecular complex is stabilised by 38.237kcal/mol which is in-between the stabilisation energies of the corresponding bimolecular structures, Alg-Gly and Alg-Ba^{2+}, demonstrating the conformational stability and compatibility of the three molecules in conjugation with each other. Interestingly and unexpectedly, all the energy values viz., bonding and non-bonding energies underwent stabilisation with all ΔE values in negative (Table 5.5), further confirming the thermodynamic and steric rationality of the selected plasticiser and crosslinker combination for alginate fibres.

Table 5.5: Calculated energy parameters (kcal/mol) of polymer-plasticiser, polymer-crosslinker and crosslinked-plasticised polymer assemblies

Name	*ΔEnergies*[a]						
	ΔTotal[b]	*ΔBond*[c]	*ΔAngle*[d]	*ΔDihedral*[e]	*ΔVdw*[f]	*ΔH-bond*[g]	*ΔElectro*[h]
Alg-Gly_5[i]	-66.183	0.264	-1.142	3.125	-15.197	-1.389	-51.837
Alg-Ba^{2+}_{10}[j]	-35.587	0.458	-1.417	6.321	5.654	0.000	-46.602
Alg-Gly_5-Ba^{2+}_{10}[k]	-38.237	-0.251	-4.621	-13.032	-13.616	-1.391	-5.321

[a] $\Delta E_{binding}$ = E(Host.Guest) - E(Host) - E(Guest)

[b] Total steric energy for an optimised structure

[c] Bond stretching contributions

[d] Bond angle contributions

[e] Torsional contribution

[f] van der Waals interactions

[g] Hydrogen-bond energy function

[h] Electrostatic energy

[i] Five glycerol molecules in conjugation with an alginate chain

[j] Ten Barium ions in conjugation with an alginate chain

[k] Five glycerol molecules and ten barium ions in conjugation with an alginate chain

5.4.8.2. Tensile analysis of crosslinked-plasticised-alginate fibres

Molecular mechanics simulations of polymer-plasticiser-crosslinker composite, wherein the energy changes calculated involved in the amalgamation of alginate, glycerol and barium cations, derived energy relationships based on molecular interactions and thus predicted the effect of formulation variables on Young modulus and ultimate strain of the composite material. Conceptually, the addition of a plasticiser may lead to lowering of rigidity of the polymer matrix thereby lowering the elastic modulus and increasing in ultimate strain as was evident from the experimental results and is in line with previous studies (Gil *et al.*, 2006). Additionally, with the inclusion of chemical and physical cross-links, increases the Young modulus and hence decreases the ultimate strain of a crosslinked composite as predicted in the experimental results and similar studies (Griebel *et al.*, 2005). It is therefore proposed that the mechanical properties of the alginate fibres may be predominantly dependent on the cohesive energy density (CED) of/between the polymer fragments based on the various intra- and inter-molecular interactions mainly in terms of non-bonded energy terms such as van der Waals forces (vdWf).

A typical relation between Young's modulus (E) and CED was derived by Roberts *et al.* (1991) as follows:

$$K = x.\frac{E_{coh}}{V} \qquad \textit{Equation 5.14}$$

$$CED = \delta^2 = \frac{E_{coh}}{V} \qquad \textit{Equation 5.15}$$

$$K = \frac{E}{3(1-2\nu)} \qquad \textit{Equation 5.16}$$

where, K is bulk modulus, E_{coh} is energy of vaporization ($J.mol^{-1}$), V is the molar volume ($cm^3.mol^{-1}$), δ is the solubility parameter, ν is Poisson's ratio and x is a constant related to the cubic lattice of the molecule under investigation.

From Equation 5.14-5.16,

$$E \propto CED \qquad \textit{Equation 5.17}$$

The complexation of 10-monosaccharide units' alginate chain with five glycerol molecules led to a decrease in vdWf by ~15kcal/mol (Table 5.5) which in turn may lead to a decrease in CED thereby decreasing the Young's modulus. This seems obvious as a reduction in cohesion will help the adjacent polymer chains to stretch/slide over each other thereby increasing the elongation of the fibres. Apart from this, the water soluble plasticiser, glycerol, used in this investigation showed a high affinity to diffuse into and interact with the polymer molecules (as shown in Figure 5.14 and total energy stabilisation) by forming non-bonding interactions in terms of hydrogen bonding contribution, vdWf and electrostatic interaction (the ΔE of all these energies is negative; Table 5.5) and hence lead to an increasing in polymer's mobility. In this way, the brittle character of the polymer may decrease with an increase in plasticiser's concentration (Gutiérrez-Rocca and McGinity, 1994). The increase in ultimate strain with the addition of a plasticiser can also be explained based on above data and discussion.

Coming to the prediction of the effect of crosslinking on tensile properties of alginate fibers, there have not been many fully atomistic simulations of crosslinked polymers in which tensile properties have been calculated as a function of energy values of the complexed molecular structure. Although the total energy was stabilised by ~36kcal/mol in Alg-Ba^{2+}, an increase of vdW energy by 5.654kcal/mol (Table I) was observed which may be responsible for the increase in the Young's modulus due to an increase in CED with increase in barium ions concentration. Apart from dispersion forces, Alg-Ba^{2+}_{10} was not stabilised by H-bonding and even the electrostatic were less stabilised, as compared to Alg-Gly_5.

Griebel *et al.* (2005) suggested a logarithmic dependency of the Young's modulus on the amount of cross-links and deduced the following relationship:

$$P(r) = E_0 + \alpha \ln(r) \qquad \textit{Equation 5.18}$$

where, P was a estimator to predict Young's modulus, α was a constant and E_0 was a parameter with units of GPa. Furthermore, in accordance with the Lennard-Jones energy potential, the elastic modulus is a measure of the rigidity of materials and is directly related to the energy of interaction between molecules and their distance of separation (Roberts *et al.*, 1991). The simulation results showed that ionic cross-links may cause disturbance at high crosslink densities resulting in distortion of bond lengths, angles, and torsions from their equilibrium values eventually causing strained and rigid-framework. Additionally, the buildup of cohesion between adjacent polymer chains (intermolecular crosslinking) may initiate a significant axial stress providing a reasonable explanation for the experimentally observed reinforcement and rigidification of the crosslinked formulations leading to decreased ultimate strain.

The effect of variation of levels of plasticiser and crosslinker on the tensile properties of alginate fibres can be explained on similar lines. The CED in case of Alg-Gly_5-$Ba^{2+}{}_{10}$ seems to be tilted more in favour of Alg-Gly_5 with ΔVdw of Alg-Gly_5-$Ba^{2+}{}_{10}$ (~13kcl/mol) close to that of Alg-Gly_5. An increase in crosslinking may significantly decrease the liquid (plasticiser) transport through the matrix (by regidification) and may even push out the already present glycerol (diffused within the matrix) thereby hindering the impact of plasticiser (by reinforcement). In the opposite way, an increase in plasticiser content may result in weakening of Ba^{2+} induced intermolecular attractions thereby increasing the polymer's free volume causing an increase in their flexibility by allowing the polymer molecules to move more freely (Gutiérrez-Rocca and McGinity, 1994). Nevertheless, the effect of addition of Ba^{2+} on Alg-Gly_5 was evident from the fact that all the energy values attained stabilisation with even stretching and torsional contributions playing their part in structural optimisation. This confirms that an optimised alginate fibre formulation having desired tensile properties can be attained by varying the concentrations of plasticiser and crosslinking agent.

5.4.8.3. Tensile analysis of drug-loaded optimised crosslinked-plasticised-alginate fibres

The mechanically optimised fibres were loaded with model drugs, diclofenac and ciprofloxacin. The addition of individual drugs significantly affected the tensile properties of alginate fibres with a steep decline in Young's modulus. However, the alginate fibres regained the Young's modulus' original magnitude when both the drugs were incorporated simultaneously (and cumulatively) into the optimised formulation (Table 5.4). A molecular

mechanics assisted model building and energy refinements approach to establish the mechanism for the above interesting observations.

The results obtained when loading fibres with either ciprofloxacin or diclofenac drug were in line with the previously reported work by Wu and McGinity (1999) where they used chlorpheniramine maleate and ibuprofen to influence the mechanical properties of polymeric films and proposed that these drugs acted as “non-traditional plasticisers” and hence caused a reduction in Young’s modulus. This effect is also evident from energy Equations 5.3-5.7 and Table 5.6 that the incorporation of ciprofloxacin and diclofenac into alginate accompanied with ΔvdWf of 12.679kcal/mol and 13.223kcal/mol, respectively, thereby causing a decrease in CED between the monosaccharide residues. The drug molecules seem to lie between the monosaccharide units thereby connecting them providing the required flexibility for sliding over. This decrease in CED seems to be responsible for the decline of Young’s modulus on the same ground as explained previously with addition of glycerol. Furthermore, as for the addition of glycerol, addition of drugs individually to the polymer matrix resulted in stabilisation of total steric energy of the polymer-drug complex confirming the thermodynamic stability of the molecular system – a prerequisite for efficient plasticisation.

Surprisingly but interestingly, the simultaneous incorporation of ciprofloxacin and diclofenac led to a highly increased vdWf and total steric energies (both positive in magnitude) along with highly stabilised bond angle and torsional contributions (both negative in magnitude) as calculated in Table 5.6. The geometrical conformation displayed in Figure 5.15 confirms the close interaction profile of this trimolecular complex system. The unexpected rise of Young’s modulus in the experimental results may be attributed to following:

1. Incorporation of both the drugs simultaneously (200mg ciprofloxacin + 500mg diclofenac sodium) may have led to an increase in individual drug concentration above their saturation solubility in the adhesive layer of polymeric matrix. Additionally, from Equation 5.15, CED is directly proportional to the solubility parameter. The solubility parameter (δ) may reach its maximum value after achieving saturation solubility which in turn may cause a very high increment in CED (CED $\propto \delta^2$) thereby leading to higher Young’s modulus than the individual drug’s incorporation (Roberts *et al.*, 1991).

2. An increase in cumulative drug concentration may also lead to filling up of pores leading to a decrease in porosity of the polymer matrix. According to Spriggs' equation (Spriggs,1961):

$$E = E_0\, exp(-bP) \qquad \textit{Equation 5.19}$$

where, E is the Young's modulus, E_0 is the Young's modulus at zero porosity, P is the porosity and b is a constant in Spriggs' equation. It is clear from the above exponential relationship that a decrease in porosity will lead to an increase in Young's modulus as observed in the experimental results.

3. These explanations however, do not fully define the reason for the increase in Young's modulus as once the saturation solubility is reached, the precipitated drug crystals (superfluous) may cause filling of the existing cracks, notches, voids or crazing in the polymeric fibre being tested leading to a increase in rigidity and decrease in movability of the adjacent alginate chains finally leading to a increase in Young's modulus (Gutiérrez-Rocca and McGinity, 1994). Additionally, increase in the crystallinity of a fibre structure may promote intermolecular forces, thus increasing the rigidity and brittleness of the film (Wu and McGinty, 1999).

The above three reasons may be responsible for the regaining of the Young's modulus value (from individual to simultaneous addition of drugs) thereby retaining the tensile properties of the initially optimised crosslinked-plasticised-alginate fibres by acting as "non-conventional biomedical tensile modifiers of polymeric fibres".

Table 5.6: Calculated energy parameters (kcal/mol) of polymer-drug assemblies

Name	*ΔEnergies*[a]						
	ΔTotal[b]	*ΔBond*[c]	*ΔAngle*[d]	*ΔDihedral*[e]	*ΔVdw*[f]	*ΔH-bond*[g]	*ΔElectro*[h]
Alg-Cpr[i]	-20.652	0.001	-1.683	0	-12.679	0.215	-6.605
Alg-Dcl[j]	-12.447	-0.123	1.385	-1.554	-13.223	0.961	0.109
Alg-Cpr-Dic[k]	309.459	216.016	-44.738	-24.838	109.283	3.2408	50.4996

[a] $\Delta E_{binding}$ = E(Host.Guest) - E(Host) - E(Guest)

[b] Total steric energy for an optimised structure

[c] Bond stretching contributions

[d] Bond angle contributions

[e] Torsional contribution

[f] van der Waals interactions

[g] Hydrogen-bond energy function

[h] Electrostatic energy

[i] Ciprofloxacin molecule in conjugation with an alginate chain

[j] Diclofenac molecule in conjugation with an alginate chain

[k] Ciprofloxacin and diclofenac molecules in conjugation with an alginate chain

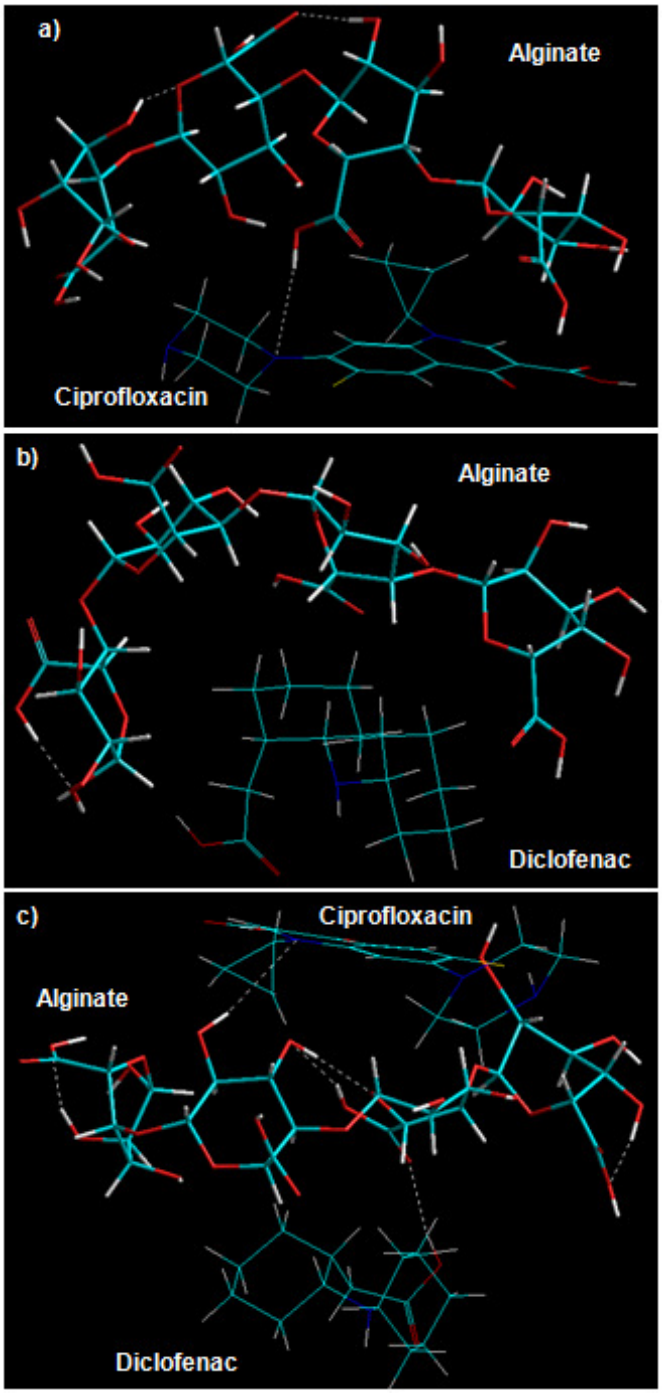


Figure 5.15: Visualisation of energy minimised geometrical preferences of alginate (4 monosaccharide units) with a) ciprofloxacin molecule; b) diclofenac molecule; and c) ciprofloxacin and diclofenac molecules, showcasing the intra- and inter- molecular interactions in drug-loaded crosslinked-plasticised-alginate fibres after molecular simulations in vacuum. Colour codes for elements: C (cyan), O (red), N (blue), and H (white)

5.5. Concluding Remarks

Optimisation of the PFD in Chapter 4 provided a formulation which necessitated further evaluation to determine its appropriateness in the treatment of PD. In this chapter analysis of the optimised formulations physicomechanical and physicochemical parameters revealed information regarding the degree of crosslinking, the effect of the plasticiser and the interaction of formulation components affecting the strength, flexibility and drug release from the matrix. Literature has attributed these changes to the monomeric composition of polymer and the crosslinker ion present (Martinsen *et al.*, 1987; Kuo and Ma, 2001; Tønnesen and Karlsen, 2002; Jejurikar *et al.*, 2011) as well as the interactions with the plasticiser (Avella *et al.*, 2007; Olivas *et al.*, 2008).

Evaluation of drug release using a UPLC proved to be a more sensitive method of detecting both diclofenac and ciprofloxacin release in PBS pH 4 and 6.8. Release of ciprofloxacin and diclofenac sodium was dependent on the solubility of the drug within dissolution media as well as swelling and relaxation of the polymeric matrix. Simulated molecular modelling elucidated the effect of formulation components, alginate, barium ions and glycerol, on the Young's modulus. The tensile properties of the fibre when both ciprofloxacin and diclofenac were incorporated led to higher Young's modulus than expected. Molecular modeling helped to explain this result, with hypothesised reasons relating to porosity and drug solubility saturation. Changes in vibrational energies characteristic of crosslinked alginate were observed in the IR spectra evident of the chemical changes occurring within the fibre affecting its structural characteristics. Addition of ciprofloxacin and diclofenac sodium seemed not to affect the chemical structure of the fibre. The fibre swelled considerably at pH 6.8 over the first 24 hours, however, it eroded at a steady rate for the reminder of the 10 days. In comparison, the fibre did not swell at pH 4 though it was consistently eroded over the same time period. Thus, the *in vitro* results of the fibre were promising, demonstrating the PFD's potential in the treatment of PD.

CHAPTER 6

IN VITRO ANTIMICROBIAL EVALUTION OF THE POLYMERIC FIBRE DEVICE

6.1. Introduction

An extensive range of microflora both anaerobic and aerobic, are present within the buccal cavity with over 500 species cultured from dental plaque (Pihlstrom *et al.*, 2005). Periodontal pathogens are divided into categories based on the weight of evidence available as illustrated in Figure 6.1. Teles *et al.* (2006) stated "The best way to control these periodontal infections is to control the pathogenic species residing in these locations", which forms the principle for the use of antimicrobials in the treatment of PD. Researchers in turn need to develop methods to assess the drug delivery device, confirming antimicrobial activity of the formulation against periodontal pathogens *in vitro*.

Since 1929, antimicrobial susceptibility tests have been used to determine the antimicrobial effect of an agent/compound placed in a medium which is able support the growth of the microorganisms (Poupard, 1994). Antimicrobial susceptibility tests may be employed in the development of new antimicrobial agents as well as investigating the efficacy of drug delivery device loaded with antimicrobial agents. Numerous screening tests have been used to determine the *in vitro* efficacy of locally placed devices against periodontal pathogens (Elkayam *et al.*, 1988; İkinci *et al.*, 2002; Yue *et al.*, 2004; Moulari *et al.*, 2006).

Elkayam *et al.* (1988) tested the *in vitro* antimicrobial effect of ethylcellulose films loaded with minocycline. Films were placed on blood agar plates which were inoculated with *Staphylococcus aureus* and incubated at 37˚C. The zone of inhibition was recorded on day 2, after which the film was transferred onto a freshly seeded agar plate. This process was repeated on day 4, 7 and 14. This screening test not only provided information regarding the antimicrobial activity of the sustained delivery device, but confirmed that the biological activity of the drug had been maintained once incorporated into a polymeric matrix.

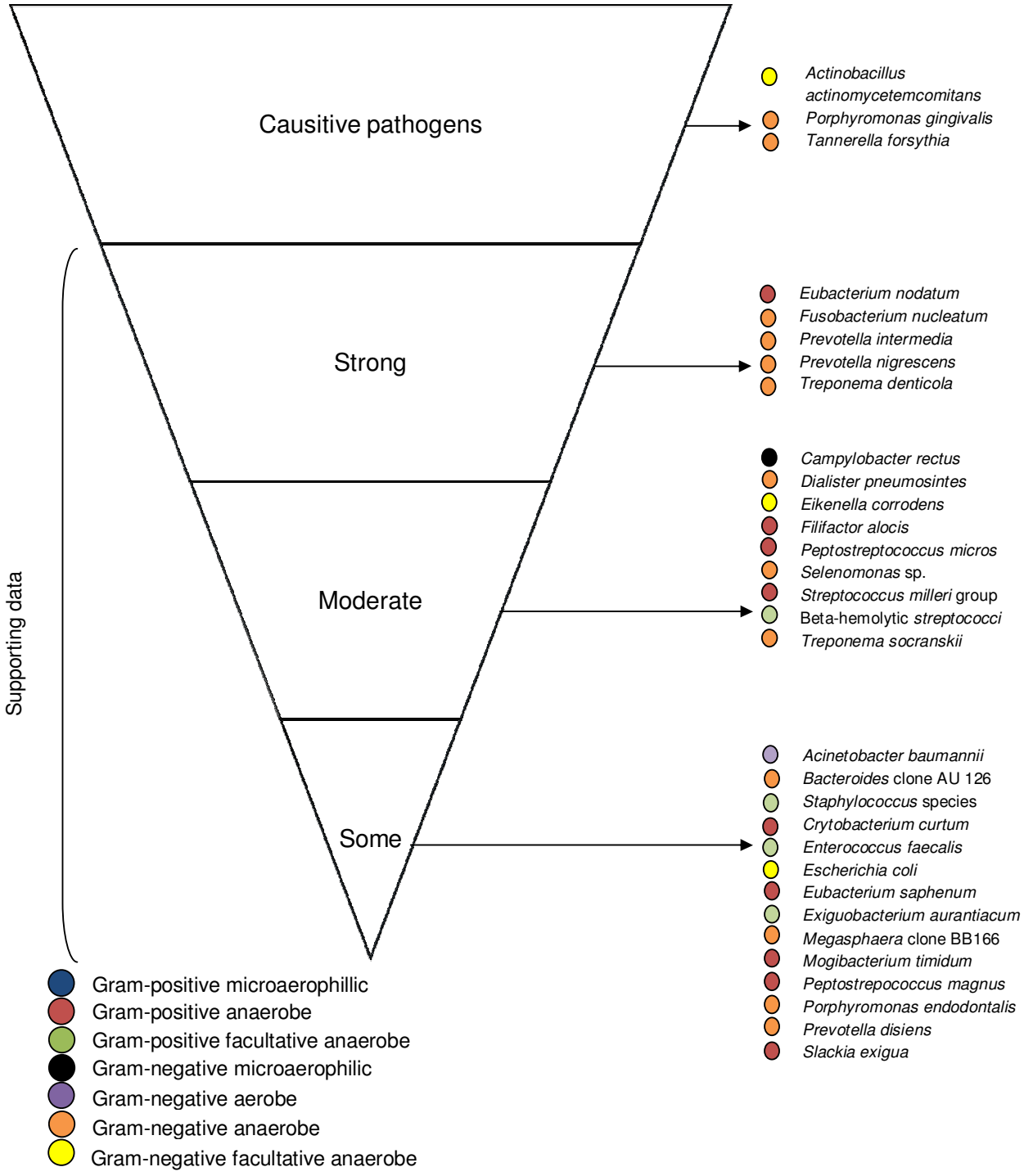


Figure 6.1: Periodontal pathogens divided into categories based on prevalence of these pathogens in PD according to literature reviewed by American Academy Periodontology (2004) and Teles *et al.* (2006)

Using a similar method, İkinci *et al.* (2002) investigated the antimicrobial activity of chitosan against *P. gingivalis*. Variations in the formulation such as molecular weight of chitosan, degree of deacetylation and incorporation of chlorhexidine were compared against one another detecting changes in antimicrobial activity by measuring the zones of inhibition. The microbial susceptibility tests assisted in the identification of the necessary components in the development of an optimal formulation measuring the zone of inhibitions on Schaedler agar, supplemented with hemin and vitamin K, and inoculated with *P. gingivalis*, isolated from subgingival plaque.

Bacteriological studies may be utilised in determining whether *in vitro* dissolution release correlates to effective inhibition of periodontal pathogens as described by Yue *et al.* (2004). The study tested the antimicrobial activity of chlorhexidine-loaded microspheres, which were compressed into a chip, against *P. gingivalis* and *B. forsynthus*. The polymeric chip was placed on plates, which were inoculated with pathogens and the zone of inhibition was measured over 10 days.

In vitro antimicrobial tests have further proved their usefulness as screening assays to determine the activity of new compounds or devices against a range of periodontal pathogens. The bactericidal activity of *Harungana madagascariensis* Lam. ex Poir. ethyl acetate leaf extract was compared to ethyl acetate extract-loaded nanoparticles (Moulari *et al.*, 2006). The minimum bactericidal concentration was determined using the microtiter plate method, where bacterial strains were combined in a well with either the leaf extract or the extract was loaded in nanoparticles. Analysis of the data compared the antimicrobial activity of the leaf extract to the extract-loaded nanoparticles against the *Actinomyces*, *Fusobacterium*, *Lactobacillus*, *Prevotella*, *Propionibacterium* and *Streptococcus* species.

The aforementioned studies highlight the importance of antimicrobial assays in the *in vitro* analysis of controlled delivery devices placed within the gum pocket for the treatment of PD. They served as the basis for incorporation of similar antimicrobial analysis in this study.

Ciprofloxacin was chosen as the model antimicrobial drug for incorporation within the PFD. This fluoroquinolone antibiotic has been used both systemically and locally in the treatment of periodontal diseases. It is a broad spectrum bactericidal antibiotic, which targets bacterial DNA, specifically topoisomerase II enzyme. Chin and Neu (1984) compared activity of ciprofloxacin to norfloxacin, cefotaxime, cephalexin, ceftazidime, moxolactam, amoxicillin and methacillin against a number of microorganisims. Their results confirmed ciprofloxacin inhibits both anaerobic and aerobic cocci and bacilli in relatively low concentrations (≤0.05µg/ml) as well as demonstrating consistently better activity compared to norfloxacin. Furthermore, ciprofloxacin inhibited gentamicin-, ameikacin-, cefotaxime-, and moxalactam-

resistant members of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus.*

An *in vitro* study conducted on bacteria invading the oral cavity investigated ciprofloxacin's activity against causative pathogens (Wade, 1989). The antibiotic was dissolved in distilled water preparing a stock solution ($0.2\%^{w}/_{v}$) and diluted in Wilkins-Chalgren agar, in 9cm petri dishes, at a final concentration of 128-0.25mg/L. Once the plates were inoculated with the appropriate microorganism they were incubated for 96 hours in an anaerobic chamber and the minimum inhibitory concentration (MIC) was determined. This study quantified ciprofloxacin's activity against *Actinomyces* spp. (MIC range 0.5-128mg/L), *Bacteriodes* spp. (MIC range1-4mg/L), *Eubacterium* spp. (MIC range 1-2mg/L), *Fusobacterium nucleatum* (MIC range 2-4mg/L), *Peptostreptococcus* spp. (MIC range <0.25-16mg/L), *P. gingivalis* (MIC range 0.25-16 mg/L) and *Selenomonas* spp. (MIC range <0.25-0.5mg/L). The MIC range and values against *Actinomyces* spp. confirm that *in vitro* ciprofloxacin is needed in higher concentrations to inhibit growth of this microorganism. However, *in vivo* ciprofloxacin demonstrates better activity against *A. actinomycetemcomitans* than expected. Easmon and Crane (1985) confirmed that ciprofloxacin is four to eight times more concentrated within human neutrophils compared to extracellular levels.

Significantly higher levels of ciprofloxacin where found in polymorphonuclear leukocytes (PMNs) *in vivo* compared to intracellular fluid (Garraffo *et al*., 1991). This explains the higher levels of ciprofloxacin in gingival crevicular fluid compared serum levels (Tözüm *et al*., 2004). PMNs, released as part of the primary host defense, are responsible for the phagocytosis of bacteria, however, *A. actinomycetemcomitans* seems to evade destruction via PMNs (Holm *et al*., 1993). Cacchillo and Walters (2002) investigated the effect of ciprofloxacin loaded PMNs on *A. actinomycetemcomitans*. Their study concluded that when high concentrations of bacteria to PMNs (90:1 and 30:1) is present ciprofloxacin loaded PMNs were more effective than the control PMNs or ciprofloxacin alone in killing *A. actionmycetecomitans*.

The review of the literature confirmed that ciprofloxacin has been used effectively in the treatment of PD and is active against causative periodontal pathogens. To confirm and quantify the antibacterial activity of the optimised PFD, the fibres were analysed against *Escherichia coli* (*E. coli*) (ACC 8739) and *Enterococcus faecalis* (*E. faecalis*) (ATCC 29212), representative of anaerobic Gram-negative and Gram-positive microbe respectively, as well as *Streptococcus mutans* (*S. Mutans*) (NCTC 10919), a facultative Gram-positive anaerobe. In Chapter 3, it was mentioned that *E. Faecalis* and *E.coli* have been found present in PD. *S. Mutans* has been used on numerous occasions to test the antimicrobial activity of a device formulated for the treatment of PD (Ahmed *et al*., 2009; Muchalambe *et al*., 2010; Ramachandra *et al*., 2011). These microbes serve as model test pathogens which

determined if sufficient ciprofloxacin was entrapped and then subsequently released from the PFD to in inhibit microbial growth over 10 days. Both qualitative and quantitative data was attained through agar diffusion assays and minimum inhibitory concentration (MIC) assays respectively. Ethics was not required for this project as stipulated in appendix D.

6.2. Materials

Nundron™ Surface MIC plates were purchased from Nunc, Denmark. Mueller Hinton (MH) agar was obtained from Oxoid, Hampshire. Iodonitrotetrazolium chloride indicator dye was procured from Sigma-Aldrich, St Louis, Missourri, USA and plate seals were attained from AEC, Amersham. Stock culture (*S. Mutans*) as well as sheep's blood were obtained from the National Health Laboratory Services. Other materials utilised were purchased from suppliers as described in previous chapters.

6.3. Methods

6.3.1. Preparation of fibres

The optimised PFD was prepared using a fixed combination of alginate, barium chloride and glycerol as indicated in Table 6.1. Each formulation was prepared in triplicate; (1) ciprofloxacin-loaded (200mg) fibres, (2) ciprofloxacin/diclofenac sodium-loaded (200mg/500mg) fibres and (3) drug-free fibres. Fibres were prepared as outlined in Chapter 3 Section 3.3.1 with model drugs added to distilled water followed by the addition of alginate.

Table 6.1: Composition of optimised formulations

Formulation components	*1*	*2*	*3*
Alginate ($\%^{w}/_{v}$)	3.14	3.14	3.14
Glycerol (mL)	22.54	22.54	22.54
Barium chloride 2-hydrate ($\%^{w}/_{v}$)	10.00	10.00	10.00
Ciprofloxacin (mg)	200	200	-
Diclofenac sodium (mg)	-	500	-

6.3.2. Preparation of culture and media

Guidelines obtained from the National Committee on Clinical Laboratory Service (NCCLS) (2003) were followed ensuring accurate microbiological assay and transfer techniques. Stock cultures were stored at -20˚C. Subcultures were prepared in Trytone Soya Agar (TSA), incubated at 37˚C for 24 hours and checked for purity. Pure colonies were then isolated and further subcultured in TSA. Table 6.2 contains the strains of test organisms which were used in this study. Weekly subcultures were kept for stock culture maintenance. Media was prepared according to protocol provided by the supplier. Media components were weighed, dissolved in distilled water, autoclaved (Butterworth) for 15 minutes at 121˚C and pre-incubated to confirm sterility.

Table 6.2: Microbial test organisms with corresponding Gram stains, incubation conditions and reference strains

Test organism	*Gram stain*	*Incubation conditions*	*Reference strain*
Escherichia coli (*E. coli*)	Negative	Aerobic, 37˚C for 24hr	ACC 8739
Enterococcus faecalis (*E. faecalis*)	Positive	Aerobic, 37˚C for 24hr	ATCC 29212
Streptococcus mutans (*S. mutans*)	Positive	Anaerobic (candle jar), 37˚C for 24hr	NCTC 10919

6.3.3. Agar diffusion studies

Agar plates were prepared as outlined in Chapter 3 Section 3.3.5 for plates inoculated with *E. coli* or *E. faecalis* cultures. The plates inoculated with *S. Mutans* were prepared with 100µL of culture and 20mL of a 2.5%$^{v}/_{v}$ sheep's blood in Mueller Hilton Agar. Samples of fibres were transferred onto the solidified agar using sterile forceps to prevent contamination. Ciprofloxacin/diclofenac-loaded fibres were tested simultaneously with the drug-free fibres, which served as the control (Figure 6.2a). Agar diffusion studies were conducted in triplicate for each test microbe. The plates were placed in the fridge for 1 hour, allowing the drug to diffuse from the fibre, after which they were incubated at 37˚C for 24 hours. Iodonitrotetrazolium chloride indicator dye (INT) (0.04mg/mL) solution was sprayed onto *E. coli* and *E. faecalis* inoculated plates to visualise the inhibition zones. INT in the presence of dehydrogenase changes colour to purple red. Dehydrogenase enzyme is present in metabolically active microorganisms. The zone of inhibition was measured, from the fibre to the edge where microbial growth was visible, comparing fibres loaded with ciprofloxacin and diclofenac sodium to the control. Four readings were taken as represented in Figure 6.2b around the fibre as it is an irregular shape with an asymmetrical zone of inhibition.

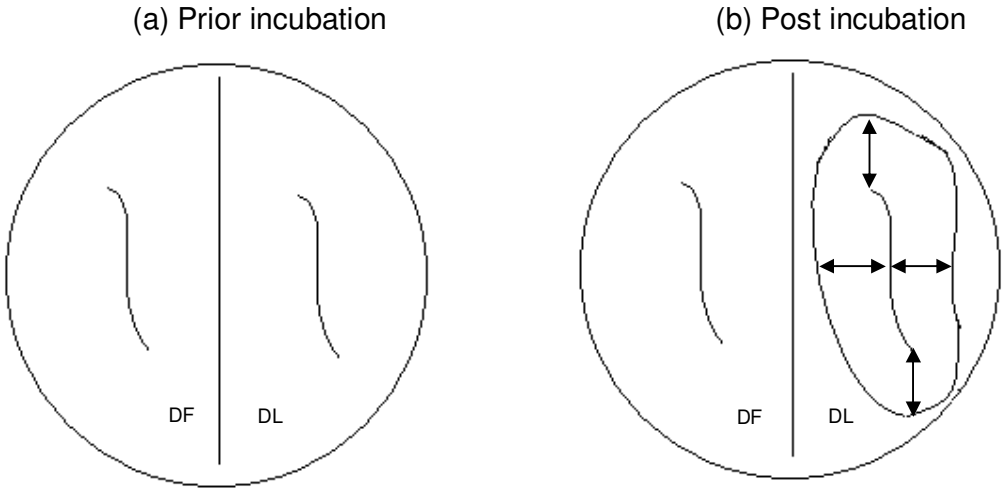


Figure 6.2: A diagrammatic sketch representative of agar diffusion plates (a) prior to incubation and (b) post incubation with arrows measuring the anticipated zone of inhibition of drug-free (DF) to that of ciprofloxacin/diclofenac-loaded (DL) fibres

6.3.4. *In vitro* dissolution studies

Dissolution apparatus used was as described in Chapter 3 Section 3.3.6. Samples were removed on day 1, 2, 4, 6, 8 and 10 withdrawing 4mL of solution which was retained at -4˚C for further antimicrobial analysis. For comparative purposes dissolution samples were collected from drug-free, ciprofloxacin-loaded as well as ciprofloxacin/diclofenac-loaded fibres in PBS pH 4 and 6.8. As previously discussed the pH range of the periodontal pocket is large and the behaviour of crosslinked alginate fibres varies considerably depending on pH.

6.3.5. Minimum inhibitory concentration (MIC) assays

The MIC micro-dilution bioassay (NCCLS, 2003) was used to quantitatively assess the antimicrobial activity of the PFD. Dissolution samples were diluted 1 in 10 with sterile water and vortexed prior to testing. Aseptically, 100µL of distilled water was added to each well of a 96 well microtitre plate, followed by a further 100µL of dissolution sample (samples from day 1, 2, etc) added to the first row of the microtitre plates. Serial dilutions were performed transferring 100µL of sample from the well above to the well below, each dilution halving the sample concentration in each well with every transfer, as in Figure 6.3. Each dissolution sample was tested in duplicate. Bacterial cultures were subcultured from stock agar plates and grown in Tryptone Soya broth overnight to confirm viability. Cultures were diluted 1:100 in fresh Tryptone Soya broth, yielding an approximate inoculums size of 1×10^8 colony forming units (CFU)/mL. Each well was further inoculated with 100µl of bacterial culture. Plates were incubated for 24 hours at 37˚C. MIC plates inoculated with *S. mutans* were placed in an anaerobic candle jar and incubated for 24 hours. Prior to incubation, the microtitre plate was sealed with adhesive seal film to prevent loss of any volatile samples.

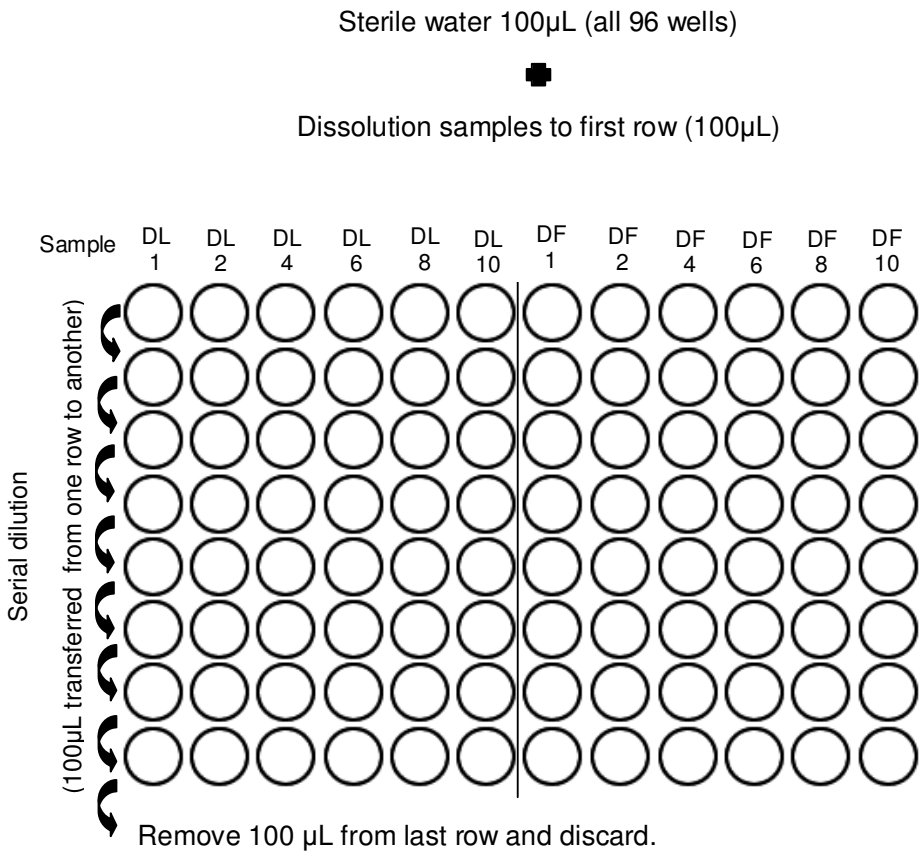


Figure 6.3: Schematic representation of MIC plate representing addition of sterile water to each of the 96 wells and dissolution samples from each sample time point added to the first row, followed by serial dilutions transferring 100µL from row to row, finally discarding 100µL from last row (DL – drug-loaded sample; DF – drug-free sample)

Negative and positive controls were included in each assay as summarised in Table 6.3. The ciprofloxacin and diclofenac controls were prepared by dissolving the drug in sterile water with final concentrations of 0.1mg/mL. Equal quantities of the solutions were combined to test if the antimicrobial activity of ciprofloxacin was affected by diclofenac sodium. INT (0.04mg/mL) solution was prepared and 40µL added to each well. After 6 hours the MIC results were read as the lowest concentration of sample to inhibit microbial growth.

Table 6.3: Controls used to verify MIC results

Positive controls	*Reason for inclusion*
Ciprofloxacin (0.01mg/mL)	To confirm activity of antibiotic used.
Ciprofloxacin (0.005mg/mL) and diclofenac sodium (0.005 mg/mL)	To verify the antimicrobial activity of ciprofloxacin is maintained when combined with diclofenac sodium.
Negative controls	*Reason for inclusion*
Drug-free fibre dissolution samples (1, 2, 4, 6, 8, 10 days)	To prove that the formulation without ciprofloxacin has antimicrobial activity.
Diclofenac sodium (0.01mg/mL)	To confirm that diclofenac sodium has no antimicrobial activity.
PBS pH 4 & pH 6.8	To ensure that the buffer has no antimicrobial activity and that it is not contaminated.
Broth (with culture)	To verify that the broth can support the growth of microorganisms.
Broth (without culture)	To validate that the broth was not contaminated.

6.4. Results and Discussion

6.4.1. Qualitative studies assessed using agar diffusion assays

Agar diffusion assays were used to investigate if the fibres contained sufficient ciprofloxacin and if the entrapped drug was released from the polymeric matrix to inhibit microbial growth. Ciprofloxacin/diclofenac-loaded fibres significantly inhibited the growth of all three test organisms while the control fibres showed no activity. The zones of inhibition are visually represented in Figure 6.4 and the mean measurements summerised in Table 6.4. The irregular zone of inhibition surrounding the fibre as well as the necessity to quantitatively measure the effectiveness of the fibre in releasing ciprofloxacin limits the use of this assay to qualitative purposes only.

Table 6.4: Mean zone of inhibition measurements for test organisms (N=3)

Test organism	*Zone of inhibition (mm)*
E. coli (ACC 8739)	14.96 ± 0.94
E. faecalis (ATCC 29212)	14.83 ± 0.32
S. mutans (NCTC 10919)	17.79 ± 2.65

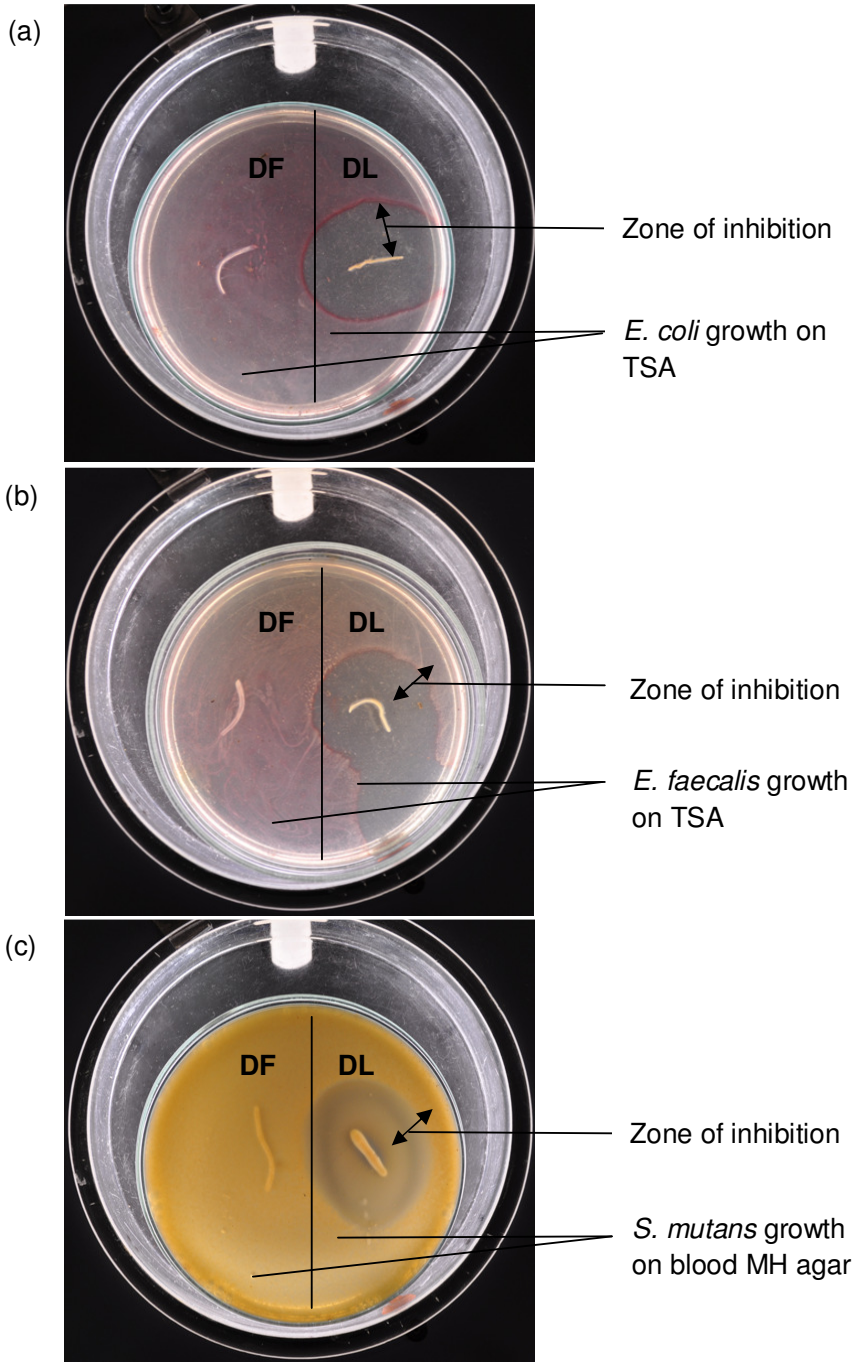
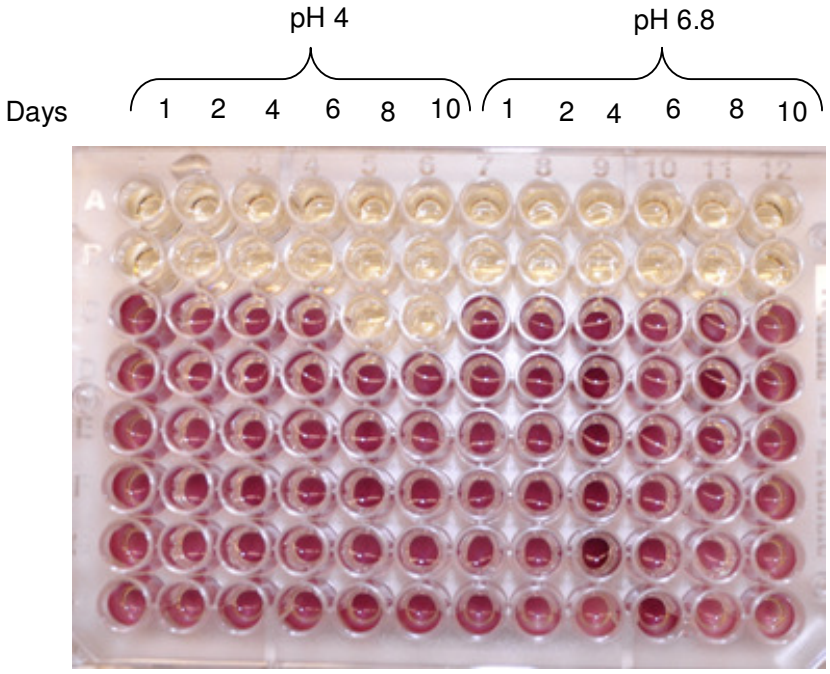


Figure 6.4: Agar diffusion assays comparing drug-free fibres (DF) to ciprofloxacin/diclofenac-loaded fibres (DL) inoculated with (a) *E. coli*, (b) *E. faecalis* and (c) *S. mutans*

6.4.2. Quantitative studies assessed using MIC assays

The purpose of performing MIC analysis on the PFD was to determine if sufficient ciprofloxacin was released from the fibre at each time point. Furthermore, it was necessary to quantify the antimicrobial activity of the prolonged delivery device against the three test pathogens that were screened for activity in the diffusion assays. The results are tabulated for comparison in Tables 6.5 and 6.6, representative of dissolution studies conducted in PBS pH 4 and 6.8 respectively. The study confirmed that sufficient ciprofloxacin was released from both ciprofloxacin- and ciprofloxacin/diclofenac-loaded fibres to inhibit growth of all three microorganisms. Therefore, the incorporation of diclofenac had no effect on the antimicrobial activity of the device. Both formulations had significantly more activity against *E. coli* (MIC range 0.1-0.7µg/mL) and *E. faecalis* (MIC range 0.2-1.1 µg/mL) compared to *S. mutans* (MIC range 0.7-2.1µg/mL). The MIC range for *E. coli* (0.1-0.4µg/mL for ciprofloxacin-loaded and 0.5-0.8µg/mL for ciprofloxacin/diclofenac-loaded fibres) and *E. Faecalis* (0.2-0.3µg/mL for ciprofloxacin-loaded fibres and 0.3-0.5 µg/mL for ciprofloxacin/diclofenac-loaded fibres) is consistent throughout the 10 day sampling period. The MIC range for *S. Mutans* (0.4-1.0µg/mL for ciprofloxacin-loaded fibres and 0.7-2.1µg/mL for ciprofloxacin/diclofenac-loaded fibres) identified some variation from sampling times but was still within the ranges for MIC testing. Consistent MIC results over the testing period attest to steady concentrations of ciprofloxacin being released from one time point to another. Figure 6.5a confirms that the MIC was maintained over the 10 days, its antimicrobial activity seems somewhat independent of the pH of the PBS, testing the fibres activity against *E.coli*. Similar results were attained when fibres were tested against *E. faecalis* and *S. mutans*, and thus only one test organism (*E. coli*) is represented in Figure 6.5 for the sake of brevity. The controls, represented in Figure 6.5b and Table 6.5, assisted in verifying the dissolution results. The PBS pH 4 and 6.8 as well as drug-free fibres demonstrated no inhibition of growth, confirming that the antimicrobial activity noted with the drug-loaded fibre is not attributed to the buffer or formulation components correspondingly. The broth showed no growth of microorganism compared to the broth inoculated with culture, which had growth throughout. This confirmed that the broth was not contaminated and it was able to support microbial growth. In comparison, the positive controls MIC values range was from 2.5-10.0µg/mL for ciprofloxacin and 18.8-50.0µg/mL for ciprofloxacin/diclofenac combination at a ratio of 1:1. MIC results for the dissolution samples over the 10 day period tested, confirmed the efficacy of the sustainable release formulation comparable to the ciprofloxacin controls. It was verified that diclofenac has no antimicrobial activity, as no inhibition of growth was noted. However, when combined with ciprofloxacin it did not inhibit the ciprofloxacin's antimicrobial activity.

(a)



(b)

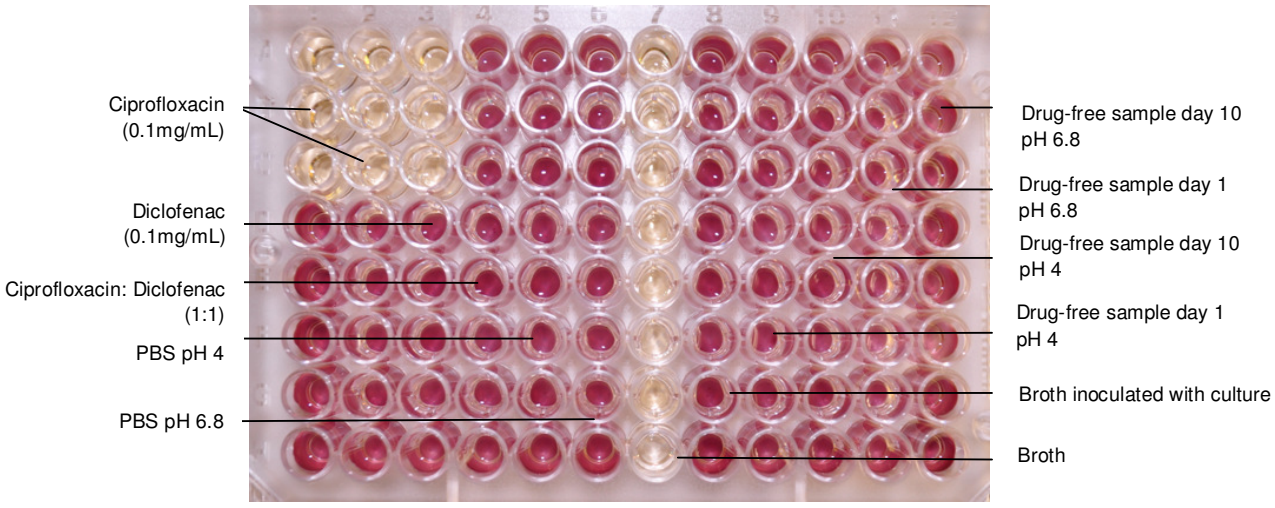


Figure 6.5: MIC plates inoculated with *E.coli*, wells which changed colour to red after the addition of INT represented growth of bacteria while wells which remained colourless represented no bacterial growth, (a) dissolution samples in PBS pH 4 and 6.8 over 10 days (b) controls used for verification of results

Table 6.5: MIC results for ciprofloxacin-loaded and ciprofloxacin/diclofenac-loaded fibres from dissolution samples in PBS pH 4 over 10 days (NI – no inhibition) (NG – no growth)

Samples	*Ciprofloxacin-loaded fibres MIC (µg/mL)*			*Ciprofloxacin/diclofenac-loaded fibres MIC (µg/mL)*		
	E. coli	*E. faecalis*	*S. Mutans*	*E. coli*	*E. faecalis*	*S. Mutans*
Day 1	0.2	0.3	0.8	0.5	0.4	0.7
Day 2	0.4	0.2	0.9	0.6	0.6	1.2
Day 4	0.2	0.4	1.0	0.7	1.1	1.4
Day 6	0.1	0.4	0.6	0.8	0.8	1.7
Day 8	0.3	0.2	0.6	0.5	0.4	1.9
Day 10	0.1	0.2	0.4	0.5	0.5	2.1
Controls:						
Ciprofloxacin	2.5	2.5	10.0	2.5	2.5	10.0
Cipro/Diclo (1:1)	18.8	25.0	50.0	18.8	25.0	50.0
Diclofenac	NI	NI	NI	NI	NI	NI
Drug free fibres (Day 1-10)	NI	NI	NI	NI	NI	NI
PBS pH 4	NI	NI	NI	NI	NI	NI
Broth with culture	NI	NI	NI	NI	NI	NI
Broth	NG	NG	NG	NG	NG	NG

Table 6.6: MIC results for ciprofloxacin-loaded and ciprofloxacin/diclofenac-loaded fibres from dissolution samples in PBS pH 6.8 over 10 days (NI – no inhibition) (NG – no growth)

Samples	*Ciprofloxacin loaded-fibres MIC (µg/mL)*			*Ciprofloxacin/diclofenac-loaded fibres MIC (µg/mL)*		
	E. coli	*E. faecalis*	*S. Mutans*	*E. coli*	*E. faecalis*	*S. Mutans*
Day 1	0.2	0.2	0.8	0.4	0.4	0.8
Day 2	0.2	0.2	0.9	0.5	0.5	1.0
Day 4	0.2	0.2	1.0	0.4	0.4	0.8
Day 6	0.2	0.2	1.0	0.3	0.3	0.6
Day 8	0.3	0.3	1.0	0.5	0.5	0.9
Day 10	0.4	0.3	1.0	0.5	0.5	1.0
Controls:						
Ciprofloxacin	2.5	2.5	10.0	2.5	2.5	10.0
Cipro/Diclo (1:1)	18.8	25.0	50.0	18.8	25.0	50.0
Diclofenac	NI	NI	NI	NI	NI	NI
Drug free fibres (Day 1-10)	NI	NI	NI	NI	NI	NI
PBS pH 6.8	NI	NI	NI	NI	NI	NI
Broth with culture	NI	NI	NI	NI	NI	NI
Broth	NG	NG	NG	NG	NG	NG

The results of this study were compared to ethyl cellulose films loaded with minocycline, whichn the methodology was discussed in Section 6.1, confirmed that the polymeric matrix itself did not have any antimicrobial activity and did not interfere with the antimicrobial activity of the loaded drug (Elkayam *et al.*, 1988). Although this type of study provided qualitative results, the MIC assays provide a quantitative analysis of the ciprofloxacin's release from the PFD over a 10 day period.

6.5. Concluding Remarks

Antimicrobial activity of the PFD loaded with ciprofloxacin was confirmed through diffusion and MICs assays. Diffusion assays proved its usefulness as a screening tool to determine if sufficient drug is incorporated within the formulation as well as confirming activity against a variety of microorganisms. Once activity was noted, it was necessary to determine if ciprofloxacin is released from the fibre is in sufficient quantities to inhibit growth of the microorganisms through assessing the dissolution samples. Furthermore, the MIC assay confirmed that the antimicrobial activity was maintained when both ciprofloxacin and diclofenac sodium were simultaneously loaded within a fibre and the antimicrobial activity was not affected by the pH of the dissolution media.

Review of the literature validated the incorporation of ciprofloxacin, as the model antimicrobial drug, within a polymeric matrix. Although this study did not test the drug-loaded fibre against causative pathogens such as *A. actinomycetemcomitans* and *P. gingivalis*, Wade (1989) verified that ciprofloxacin demonstrates activity against these causative periodontal pathogens. Due to limited access to other periodontal pathogens, it is suggested that in further research the fibres should be tested against an extensive range of microorganisms, including gram-negative anaerobes. Analysis against clinical strains of periodontal pathogens may also prove useful. In addition drug release studies in conjunction with antimicrobial studies are necessary to investigate the concentration of ciprofloxacin that needs to be released over the 10 day period.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

PD is a condition with worldwide prevalence that if left untreated it may lead to permanent tooth loss. Known risk factors in SA such as HIV, diabetes and socio-economic status affect a large portion of the South African population. This may lead to a situation where the presence of periodontal infections escalates substantially. With an already burdened health care system the need to develop innovative treatment options is clearly important. Numerous drug delivery devices are at various stages of drug development processes, with a few commercially available abroad but none of which are obtainable in South Africa. To date one study has been conducted locally, formulating tetracycline-loaded microparticles and tested *in vitro* for the treatment of PD (Govender *et al*., 2005). Therefore further research into a locally developed and manufactured drug delivery system for the treatment of PD would be highly beneficial.

Chemotherapeutic treatment of PD is normally used in conjunction with SRP and may be delivered systemically or locally. Locally drug delivery of many antimicrobial agents dominated periodontal research in the 1990's (Chapple 2009). It has been reported that local drug delivery for the treatment of PD has failed to meet the therapeutic objectives (Chappel, 2009; Fellman, 2010). At a symposium held to discuss the role of local drug delivery within the periodontal pocket, questions were raised regarding the intended patient recipients, choice of antimicrobial/antiseptic agents, use of the device in conjunction the mechanical intervention and physical characteristics of the device (Finkelman and Williams, 1998). Schwach-Abellaoui *et al.* (2000) recommended that a suitable delivery system intended for the treatment of periodontal diseases should meet the following criteria:

- The polymer must be free from impurities, additives, stabilisers, catalyst residues and emulsifies that may be eluted from the device.
- The physical, chemical and mechanical properties of the polymer should not be changed by the biological environment from a nondegradable device.
- The device should be thermally and mechanically stable.
- The device must be easily processed into the intended form i.e. film, fibre, gel, multiparticulate.
- The device should not evoke an inflammatory, toxic or carcinogenic response.
- The device should be prepared under sterile conditions or be sterilised afterwards.

These guidelines form the minimum requirements that any device should meet. The *in vitro* analysis of the device needs careful consideration to evaluate the physical and chemical properties in order for the device to meet the therapeutic requirements *in vivo*.

The majority of the research has focused on the delivery of antimicrobial agents, with little information available regarding anti-inflammatory agents used in the treatment of PD (Addy and Renton-Harper, 1996). Reddy (2003) reviewed the use of NSAIDs in the treatment of PD and concluded that the clinical trial results correlated with treatment rationale. Further research is required to determine the efficacy of NSAIDs in comparison to antimicrobials and mechanical debridement as well as determining the effect of NSAIDs delivered locally (Howell and Williams, 1993).

This study designed a local delivery device for placement within the periodontal pocket releasing ciprofloxacin and diclofenac sodium over a 10-day period. The incorporation of both ciprofloxacin and diclofenac sodium was aimed at both the microbial and inflammatory aetiology of PD. The PFD formed a strong and flexible matrix demonstrating activity against *E. coli*, *E. faecalis* and *S. mutans*. Simulated molecular models confirmed the effect of formulation components and model drug on the tensile properties of the fibre. The results thus far are promising, therefore advocating further analysis of the fibres.

7.2. Recommendations

The extremes of pH measured in the periodontal pocket impacts drug release. Most studies conducted in formulating a device have failed to test the device in an acidic and more basic environment. Incorporation of a weak acidic drug, diclofenac sodium, and a weak basic drug, ciprofloxacin, within a pH responsive polymer, sodium alginate, led to pH responsive drug release. The pH of the pocket needs to remain constant for steady drug release from the fibre. It is therefore proposed that a buffered gel, which could maintain the pH of the pocket at 6.8, could be co-administered with the fibre resulting in steady drug levels.

The major challenge posed by the study was to formulate fibres of a consistent size and shape. The force applied to the plunger of the syringe was a difficult factor to control. Ideally a syringe pump should be used where the applied pressure could be held constant. A syringe pump was purchased, however it was not suitable as the alginate solution was viscous and the flow rate required exceeded the set limits of the pump. A suitable method to ensure that homogenous fibre is formed needs further consideration.

In vitro dissolution apparatus used in testing the release properties of a local delivery device placed within the periodontal pocket vary from study to study as described in Chapter 3 Section 3.4.5. Therefore, it has not been possible compare the drug release profiles of

various devices. It is necessary to develop a suitable model to standardise dissolution studies.

Incorporation of further polymers may enhance the physicochemical and physicomechanical properties of the device. Incorporation of mucoadhesive polymers may assist in securing the fibre within the pocket. To further optimise the release properties, nanoparticles could perhaps be suspended within the fibres releasing as the matrix swells and erodes.

Antimicrobial activity of the drug-loaded fibres against the known causative periodontal pathogens, *P. gingivalis*, *T. forsythensis*, *T. denticola*, *A. actinomycetemcomitans* needs to be investigated. The fibres demonstrated excellent activity against *E. faecalis*, *E. coli* and *S. Mutans* using agar diffusion assays and MIC assays.

A suitable method for securing the device within the pocket needs further consideration. The device could be secured in place with a biocompatible cyanoacylate glue. This is the mechanism used to secure the commercially available fibre, Actiste®. Alternative methods for securing the device in place should be investigated. The proposed methods are using a biodegradable stitch or developing a bioadhesive gel.

No toxicology investigations have been conducted on the fibres as yet. Alginate and glycerol have been used extensively in formulation development. However, concern regarding barium chloride toxicity needs further investigation. Barium ions in solution may stimulate the heart muscle leading to ventricular fibrillation, which may result in death (Patnaik, 2003). The lethal dose of barium is estimated between 3-5g for an average adult (70kg) (Assimon *et al.*, 1997). Barium cations have been used in development of numerous systems intended for human administration (Zekorn *et al.*; 1992; Klöck *et al.*; 1994; Bajpai *et al.*, 2006). Although, a relatively high concentration of barium chloride was used in the crosslinking solution, the amount of cations crosslinked within the matrix needs to be determined. If the fibres were found to be toxic, the proposed solution would be to use a combination of crosslinking cations, perhaps salts incorporating zinc, aluminum and calcium ions. Furthermore, exposing the alginate crosslinked matrix to an acidic solution may retard erosion of the device (Bajpai and Sharma, 2004).

If the device was found not to have any toxic affects the next stage in the development process would be testing in a suitable animal model. Weinberg and Bral (1999) compared animal models used in evaluation of the pathogenesis and treatment modalities in PD. The review concluded that both nonhuman primates (e.g. cynomolgus monkey) and dogs (e.g. beagles) should be used as animal models with regard to periodontal research as rodent models, such as hamsters and rats, have a different dental formula.

The study resulted in the formulation of a strong yet flexible fibre capable of entrapping a high percentage of model drugs. While it is acknowledged that the further research is necessary for this device to reach the next stage of the drug development process, nevertheless it is suggested that the results represent a valuable contribution towards the successful development of a biodegradable polymeric fibre for the treatment of periodontal disease. In addition other applications of such a device warrant further investigations. Similar biocompatible drug-loaded fibres may be used as an implant, dental floss or as surgical sutures.

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APPENDICES

Appendix A:

Flexural and extensibility testing of co-blended amphiphilic triethanolamine and transesterified epichlorohydrin-based alginate fibers

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Introduction

The purpose of this study was to compare the physicochemical properties, elasticity and flexibility, of alginate fibres following the addition of various plasticizers.

Methods

Alginate fibers were prepared using a 4%$^{w}/_{v}$ alginate solution crosslinked with 2%$^{w}/_{v}$ barium chloride ($BaCl_2$). Varying concentrations (7-10%) of plasticizers namely triethanolamine (TEA) and glycerine (GLY) were added to the alginate solution to form a homogenous mixture. Fibers were left to cure for 24 hours. The resultant alginate fibres were left to dry for 48 hours and were then cut into 70mm samples for standardization. A TA.XT*plus* Texture Analyzer (TA) was used for assessing the elasticity and flexibility of the fibres. A tensile grip was calibrated at a 60mm distance from the TA sample stage. A fiber was secured to both the lower and upper custom made tensile grips during testing at a data acquisition rate of 400pps. Force-Time profiles were plotted for each fibre tested revealing the effects of each the plasticizer in the formulation.

Results

Measurement of the distance to the fibre break-point indicated the elasticity with greater distances signifying more extensible fibre formulations. The maximum Force revealed the elastic limit or tensile strength of the various fibre formulations. Formulations B, C, D and E were more extensible (4.74-19.634mm) that Formulation A (Control) (4.12mm) where no plasticizer was included. Comparison of the results revealed that Formulation D fibres were the most extensible (238.03g of Force required). The breaking-point of the fibres indicated the flexibility. The Control formulations fractured immediately on the application of force. Formulations with added plasticizers demonstrated increased flexibility. Formulation B, C, D and E did not fracture under the same force applied and were significantly more extensible.

Conclusions: Addition of TEA or GLY increased the elasticity and flexibility of the alginate fibres. Formulations comprising GLY demonstrated improved physicomechanical properties as compared to TEA formulations.

Appendix B:

Characterization of co-blended amphiphilic transesterified epichlorohydrin-based alginate fibers for oramucosal delivery

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Introduction

This study involved the formulation of polymeric fibers for the site-specific delivery of metronidazole for the management of periodontitis. Prolonged release formulations within the gum cavity may improve patient compliance, reduce gastric degradation of drug and avoid extensive first-pass metabolism.

Preparation and optimization of the metronidazole-loaded alginate fibers

A metronidazole-alginate solution was crosslinked with $BaCl_2$ into robust fibers. A plasticizer, glycerol (5-25mL), was then added to the solution as to increase the fiber flexibility. A Box-Behnken experimental design was employed to optimize the fiber formulation using various concentrations of alginate (2-4%$^{w}/_{v}$), $BaCl_2$ (0.5-10%$^{w}/_{v}$) and glycerol (5-25mL). Fifteen formulations were generated and the results obtained for three of these are presented.

Formulation	Alginate	$BaCl_2$	Transesterified epichlorohydrin
FA	2%$^{w}/_{v}$	10 %$^{w}/_{v}$	15mL
FB	4%$^{w}/_{v}$	5.25%$^{w}/_{v}$	25mL
FC	3%$^{w}/_{v}$	5.25%$^{w}/_{v}$	15mL

Determination of the fiber fracture

A Texture Analyzer (TA) determined the fiber fracture force. Samples (70mm) were placed between calibrated tensile grips 60mm from the TA sample stage. Force-Time profiles revealed the influence of glycerol on the physicomechanical properties of the fibers.

Assessment of fiber swelling and erosion

Fibers were immersed in 100mL PBS (pH4, 37°C) and analyzed every 2 days over a 10 day period. Excess buffer was blotted and samples were weighed to determine the degree of swelling. Thereafter they were dried in an oven (30°C; 48hrs) until constant mass and re-weighed to determine the extent of erosion.

Results

Textural analysis revealed that FA fractured at a lower force (0.684N) compared to FC (0.777N) due to a decreased alginate concentration (2%$^{w}/_{v}$) that compromised fiber strength. FB fractured at the lowest force (0.678N) despite the higher alginate concentration. The increased glycerol reduced the fiber strength by acting as a plasticizer. FB swelled 201.33% of the original mass which was considerably higher than FA (87.12%) and FC (167.20%) also due to the higher alginate concentration. FC had the lowest extent of erosion after 10 days (36.52%) compared to FA (25.06%) and FB (23.53%) with an increased alginate concentration and reduced plasticizer volume. Increasing the alginate concentration enhanced the quantity binding sites available for ionotropic crosslinking with Ba^{2+}, resulting in a highly interconnected and rigid fiber matrix. The addition of glycerol conversely reduced the degree of crosslinking, thus produced weaker flexible fibers through enhancement of the polymeric chain mobility.

Conclusions

Further studies are underway to assess the metronidazole release and improve the physicomechanical properties of the fiber system.

Key words: co-blended amphiphilic transesterified epichlorohydrin-based alginate fibers

Appendix C:

Evaluation of the physicochemical and physicomechanical properties of optimized ciprofloxacin- and diclofenac-loaded co-blended amphiphilic transesterified-based alginate fibers for oramucosal delivery

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To be presented at the ICPPS Conference, Academy of Pharmaceutical Sciences Young Scientists Competition, September 25-27 2011

Periodontal disease (PD), a prevalent condition worldwide, is characterized as a chronic bacterial infection with cascading inflammatory reactions that if left untreated may lead to the permanent tooth loss. The aim of the study was to design, formulate and evaluate (*in vitro*) a novel polymeric matrix system to deliver an antimicrobial and anti-inflammatory drug over 10 days for the treatment of PD. Alginate combined with glycerol was crosslinked with barium cations forming a monolithic fiber incorporating ciprofloxacin and diclofenac sodium as the model antimicrobial and anti-inflammatory agents respectively. A 3-Factor Box-Behnken Design was employed to statistically optimize the fibers according to their tensile properties and drug release. The optimized formulation (3.14%$^{w}/_{v}$ alginate, 22.54mL glycerol and 10.00$^{w}/_{v}$ barium chloride) was evaluated according to drug entrapment, drug release and hydration behavior at pH 4 and 6.8, vibrational transitions (FTIR), antimicrobial activity and tensile properties. Drug release at pH 4 occurred as a result of drug diffusing through the polymeric matrix however at pH 6.8 the disruption of the fiber structure led to drug release as a consequence of the swelling and erosion of the matrix. Ciprofloxacin was sufficiently released from the drug-loaded fibers inhibiting growth of *Escherichia coli*, *Enterococcus faecalis* and *Streptococcus mutans* over 10 days. The physicomechanical and physicochemical properties were related to the degree of crosslinking, the effect of the plasticizer and the interaction of formulation components affecting the strength, flexibility and drug release from the matrix, which may be attributed to monomeric composition of polymer and the crosslinker ion present as well as the interactions with the plasticizer. The promising *in vitro* results advocate further analysis of the fibers.

Appendix D:

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical)

Secretariat: Research Office, Room SH10005, 10th floor, Senate House • Telephone: +27 11 717-1234 • Fax: +27 11 339-5708
Private Bag 3, Wits 2050, South Africa

29 March, 2005.

Re: Deanne MG Hazle Student Number: 0101518Y

Prolonged Drug Delivery from Multi-Spheres Designed for Periodontal Disease.

This certifies that this project does not require clearance from the Human Research Ethics Committee. The study involves purely laboratory-based experimentation and no human or human tissue is involved.

Yours sincerely,

Professor Charles Feldman
Deputy Chair: Human Research Ethics Committee